Comparison of leaf hydrocarbon yields from commercial cotton, FiberMax 1320 treated with growth regulator (Stance®) vs. non-treated plants.

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ABSTRACT

The yields of hexane extractable hydrocarbons from ten plants of commercial cotton, FiberMax 1320, grown dryland in Hansford County, TX and sprayed with growth regulator (Stance®) were compared with ten plants non-treated. Significant differences were not found in leaf biomass or HC yield (as g/ weight 10 DW leaves). However, there was a significant difference (p= 0.05) in % HC yields. Plants **sprayed** with the growth regulator had a **lower** % HC yield. In contrast, plants **not sprayed** had a **higher** % HC yield. The use of a foliar spray containing both mepiquat chloride and cyclanilide (Stance®) resulted in the production decreased amounts of stored HC in cotton. It appears that Stance® not only disrupts gibberellic acid synthesis and the transport of auxins, but likely influences other synthesis pathways (including those leading to stored hydrocarbons). Published on-line www.phytologia.org *Phytologia 100(1): 1-5 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* spp., yields of hexane extractable leaf hydrocarbons, growth regulator (Stance®)

In a seminal paper, Stipanovic, Bell and Benedict (1999) reviewed the defensive role of pigment gland constituents in cotton. Cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed how gland constituents can be rapidly synthesized in response to pathogens.

Opitz, Kunert and Gershenzon (2008) examined the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They found that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments. In addition, they reported that herbivory or mechanical damage in older leaves led to terpenoid increases in younger leaves. Higher terpenoid yields were achieved by two methods: 1. increased filling of existing glands and 2. the production of additional glands. The composition of the terpenoid mixture did not significantly differ in response to herbivore, mechanical damage or jasmonic acid treatments.

Many plant species protect themselves from herbivory by a response to an attack (see Karban and Myers, 1989 for a review). It appears that early research on plant defensive chemicals focused on constitutive (or stored) chemicals such as terpenoids, tannins and aromatic metabolic compounds derived from the shikimic acid pathway (Pare and Tumlinson, 1998). But, more recently, greater focus has been on inducible plant defenses (Chen 2008; Pare and Tumlinson, 1997, 1998; Turlings, et al. 1995). Turlings et al. (1995) published a seminal paper entitled "How caterpillar-damaged plants protect themselves by attracting parasitic wasps". They showed that plants injured by herbivores emit chemical signals that attract and guide the herbivores' natural enemies to the damaged plants. Thus, indirectly, injured plants send out a "SOS" signal for help against herbivores. Pare and Tumlinson (1997) nicely documented this phenomenon in a series of experiments on cotton using beet army worms and mechanical damage to leaves.

Chen (2008) discusses that some constitutive chemicals may be increased to even higher levels after insect attack. The present research (herein) is concerned with total extractable hydrocarbons for alternative fuels and chemical feedstocks from cotton leaves.

Adams et al. (2017a) reported on the yields of pentane extractable hydrocarbons (HC) from leaves of thirty USDA germplasm cotton accessions (Hinze et al. 2016), grown with supplemental underground drip irrigation at College Station, TX. They discovered % HC yields were very high in four accessions with 11.34, 12.32, 13.23 and 13.73 % HC. Per plant HC yields varied from 0.023 to 0.172 g/ g leaf dry weight (DW). The correlation between % HC yield and average leaf DW was non-significant (0.092), suggesting that breeding for increased HC and plant biomass may be possible.

Adams et al. (2017b) conducted an ontogenetic study of a commercial cotton cultivar, (FiberMax 1320), grown under dryland conditions. They reported the dry weight of leaves reached a maximum at the 1st flower stage, and then declined as bolls opened. However, % pentane soluble hydrocarbon yields continued to increase throughout the growing season (due to the decline of leaf DW). It seems likely that as the bolls mature and seed are filled, carbohydrates from the leaves are catabolized and sugars are transported to the bolls for utilization. Per plant HC yields increased from square bud stage to 1st flower, remained constant until 1st boll set, then declined at 1st boll-opened stage. This seems to imply that most of the HC are not catabolized and converted to useable metabolites for filling bolls and seeds.

Adams et al. (2017b) also reported on four accession of cotton (SA-1181, 1403, 1419, and 2269) that were grown both in field conditions and a greenhouse to compare the environmental effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves) under natural and controlled (protected) conditions. Leaf biomass was similar but higher in two field cultivated accessions. All four accessions produced higher % HC yields under field conditions, with greenhouse plants producing lower yields ranging from 20.6 to 46.0% as much HC as found in naturally grown plants. Total HC yields (g HC / g 10 leaves) were even lower in the greenhouse with yields being only 19.7 to 39.1% as high as from field grown plants. Overall, the environmental component to the yield of free HC in cotton leaves was a major factor, with the genetic component contributing to less than half (46%) of the HC yield. This trend corresponds to literature reports of large induction of defense chemicals in cotton upon attack by herbivores and diseases.

Cotton is a subtropical, perennial, woody plant and as such, has an indeterminate growth pattern. However, in most of the world it is grown as a short life cycle "annual". Excessive vegetative growth can be detrimental as fruit may abort, crop maturity can be delayed leading to a reduced harvest (Jost, et al. 2006).

Plant Growth Regulators (PGRs or GRs) are very widely used in the cultivation of cotton (Rademacher 2015; Jost et al. 2006; Dodds, et al. 2010). PGRs are applied to balance vegetative and reproductive growth. PGRs are used to control excessive vegetative growth (PIX, mepiquat chloride; mepiquat pentaborate; cyclanilide, Stance® (cyclanilide+mepiquat chloride), etc., Rademacher 2015). PIX works by inhibiting cyclases (Rademacher 2015) involved in the synthesis of gibberellic acid (GA), thence leading to loss (decrease) of GA based compounds (eg. GA3, GA4, etc.). GAs promote longitudinal growth in plants (among other factors) (Rademacher 2015). Cyclanilide inhibits transport of natural auxins, and thus, reduces growth in cotton (Rademacher 2015).

In addition, PGRs (eg. Finish®, FreeFall®, Ginstar®, etc.) are used to defoliate cotton to aid harvesting and boll opening.

As an effort to understand the induction of defense chemicals in cotton, we present the results of a field comparison of the effect of spraying a growth regulator (Stance®) on commercial cotton, FiberMax 1320.

MATERIALS AND METHODS

Plant Materials: FiberMax 1320, dryland, dark, loam soil, JP TeBeest Farm, 36° 25' 0.6" N, 101° 32' 17.3" W, 3258 ft., Oslo, TX, avg. annual rainfall, 19.3". Ten plants were randomly selected, tagged and a plastic bag was placed over the plants to shield them from an application of growth regulator (Stance®, Bayer CropScience, Inc., Cyclanilide [1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid, 2.1%], Mepiquat chloride [N,N-dimethylpiperidinium chloride, 8.4%], 2 oz. / acre, July 1, 2017. The bags were removed ca. 5 hr after the field was sprayed. Two weeks later (July 16, 2017), ten (10) mature leaves were collected from each of the 10 protected plants and 10 leaves from 10, random plants in the sprayed field. All plants had flowers and a few young bolls. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). Three grams of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane. The jar was sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan, and the hexane was evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. All shaker extraction yields were adjusted to oven dry wt (ODW) by a correction factor (ODW) of 1.085. For the cultivated cotton from Oslo, TX, the shaker yields were corrected by the increased soxhlet extraction efficiency (SEE = x 1.14), for a Correction Factor CF = 1.24 (ODW 1.085 x SEE 1.14 = 1.24). Statistical analyses (means, variance, standard deviation, standard error of mean) were performed by use of EasyCalculation (https://www.easycalculation.com/statistics/standard-deviation.php) and t-tests were performed at socscistatistics.com

RESULTS

Biomass and hydrocarbon (HC) yields for plants sprayed with Growth Regulator (GR) and not sprayed are given in Table 1. No significant difference was found for leaf biomass nor for HC yield (as g/ weight 10 DW leaves. However, there was a significant difference (p= 0.05) for % HC yields. Plants **sprayed** with the growth regulator had a **lower** % HC yield. In contrast, plants **not sprayed** had a **higher** % HC yield.

Because Stance® contains both mepiquat chloride and cyclanilide, each of which acts on different biosynthesis mechanisms (Rademacher 2015), it is probablematical to speculate too much. However, mepiquat chloride is known to inhibit cyclases (Rademacher 2015). It seems likely that other synthesis pathways are disrupted (including those leading to stored, hydrocarbons). If so, this could explain the lower amount of HC in the plants treated with GR (Table 1). Likewise, because cyclanilide inhibits the transport of auxins (Rademacher 2015), it may also influence the pathway(s) leading to the synthesis of HC.

Table 1. Comparison of leaf biomass and HC yields for greenhouse versus field grown cotton.
GR = Growth Regulator (sprayed on plant). ns = non-significant (at p = 0.05), * = significant (at p= 0.05).

	DW 10 lvs/ plant		% HC yield		HC g/ 10 DW lvs	
Material	with GR	no GR	with GR	no GR	with GR	no GR
FiberMax 1320	5.34 g 0.261	5.38 g 0.484	**3.724%** 0.133	**4.277%**, 0.264	0.200 g 0.0115	0.234 g 0.0284
	t-test: p = 0.89 **ns**		**t-test: p=0.025 ***		t-test: p=0.106 **ns**	

However, because Stance® suppressed new vegetative growth and favors shorter internodes with few total number of main stem nodes (Jost et al. 2006), the application of Stance® may have merely slightly modified the life cycle of cotton increasing its maturation, and, perhaps signaling the plant to develop the bolls. This shift in life cycle may have led to increased conversion of HC resources in the leaves for use in the developing bolls (Adams et al. 2017b).

These results are illustrated in Figure 1, where one can see the small (non-significant) effects on leaf biomass (g DW 10 lvs) and a significant effect on % HC yield, with the sprayed plants, having a lower amount. The g HC/ DW 10 leaves values are not significantly different, but appear larger in the not sprayed plants (Fig. 1).

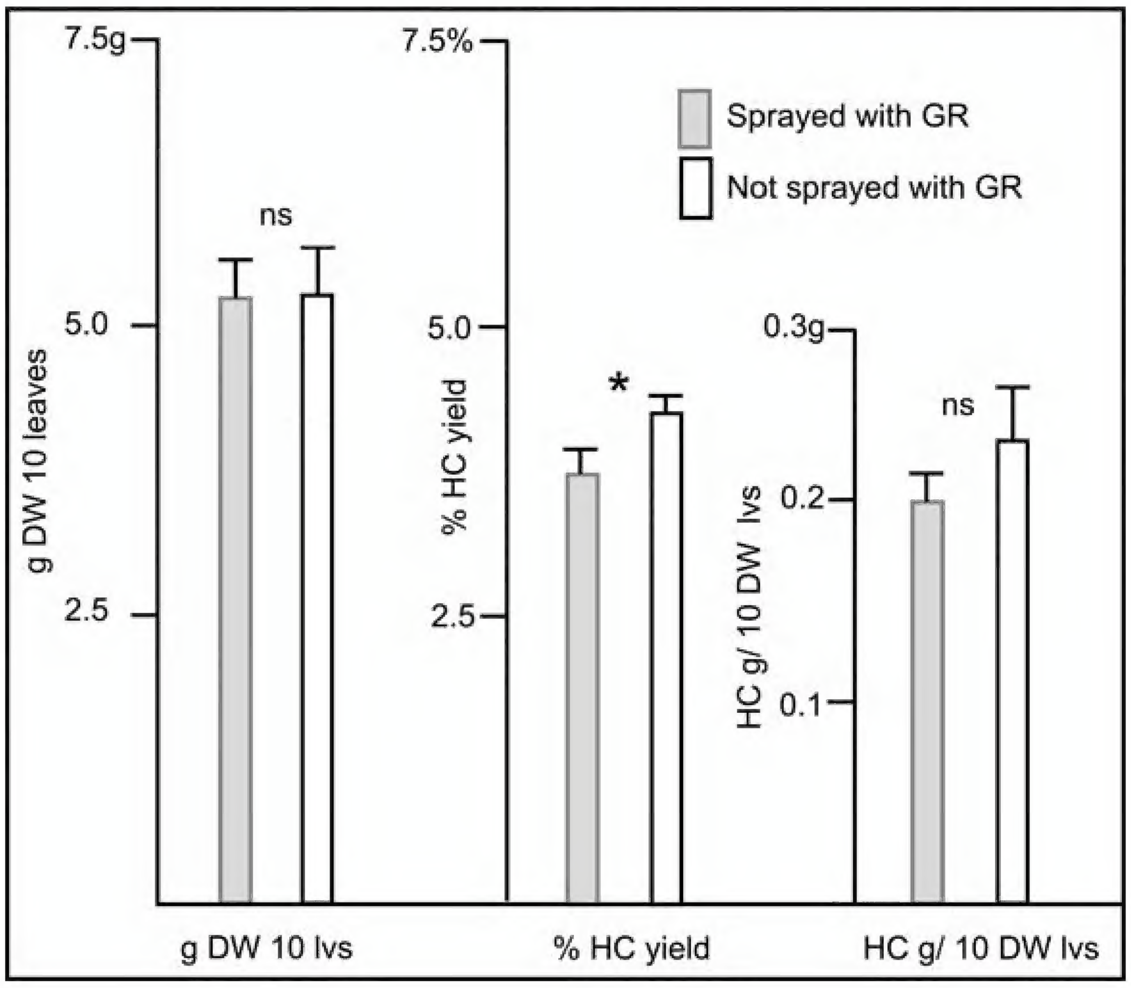

Figure 1. Bar graphs of gDW 10 lvs, % HC yield, and HC g/ 10 DW lvs for sprayed vs. not sprayed plants.

This is the first in a series of studies by our lab to determine the effects of various growth regulators (including herbicides to stress cotton plants). These results, at first, appear counter-intuitive, but upon reflection, the application of Stance®, that contains both mepiquat chloride and cyclanilide, certainly compounded the possible responses within the cotton plants. It is clear that the use of a foliar spray containing both mepiquat chloride and cyclanilide (Stance®) is not useful in promoting increased amounts of stored HC in cotton. Additional research involving a complete examination of the biomass in stems, leaves, flowers, and bolls is needed to better understand the impact of Stance® on cotton metabolism.

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Comparison of hydrocarbon yields in four cotton accessions: regular vs. limited (induced dryland) irrigation

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ABSTRACT

Four Upland accessions of cotton *Gossypium hirsutum* (SA-1181, 1403, 1419, and 2269) were grown in limited irrigation and regular irrigated field conditions to compare water stress effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves). Analyses revealed a significant difference between irrigations and significant interaction between entries by irrigation or genotype by environment (GxE) for % HC yield and total HC ($p < 0.05$). However, when entries were examined in each irrigation, no significant differences were found among entries for % yield HC, and significant differences ($p = 0.03$) were found for total HC under regular irrigation while high significant differences were found under limited irrigation or induced stress/dryland field conditions for these two traits ($p < 0.001$). The average % HC yields and HC total were significant for SA-2269 compared to SA-1181, 1403, and 1419 under stress/dryland field conditions. The percent increase in HC yields (regular to limited irrigation) were: SA-1181 18.1%; SA-1403 21.5%; SA-1419 30.3% and SA-2269 42.1%. HC yields (as g HC/leaf DW) were either barely significant (SA-1181, p=0.037*) or non-significant (SA-1403, SA-1419), except for SA-2269, that was very highly significant due to the fact that the biomass was almost as large in limited as in regular irrigated plots and % HC yield was much greater than in regular irrigated plots. These results clearly show that the yields of HC in cotton are higher for these accessions when grown under water stress. In addition, there are some differential responses among the four accessions, with SA-2269 showing the greatest increase in HC yields in induced stress/dryland conditions. Published on-line www.phytologia.org *Phytologia 100(1): 1-5 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* spp., yields of hexane extractable leaf hydrocarbons, stress induction, dryland, irrigated

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Cotton (*Gossypium* spp.) is a perennial shrub / tree that is grown as an annual crop in most of the world and our cultivated varieties have an unusual evolutionary history (Wendel and Grover, 2015). Even though cotton is the most important renewable natural fiber (Ulloa et al. 2007), alternative sustainable, renewable sources of petrochemicals and fuels from arid and semi-arid land as the cotton crop may be another source of income for producers. Screening 614 taxa from the western US for their hexane soluble hydrocarbon (HC) and resin (methanol soluble) yields, Adams et al. (1986) found the highest HC yielding species were from arid and semi-arid lands in the Asteraceae (11 species), Asclepiadaceae (1), Celastraceae (1), Clusiaceae (1) and Euphorbiaceae (1). The top 2% (12/614) had whole plant HC yields ranging from 10.4 to 16.4%.

Recently we reported (Adams et al. 2017a) on the yields of pentane extractable hydrocarbons (HC) from leaves of thirty USDA germplasm cotton accessions (Hinze et al. 2016), grown with supplemental underground drip irrigation at College Station, TX. We discovered % HC yields were very high in four accessions: SA-1181 12.32%; SA-1403 9.08%; SA-1419 13.23% and SA-2269 11.09% HC. Per plant HC yields varied from 0.119 to 0.178 g/ g leaf dry weight (DW). The correlation between % HC yield and average leaf DW was non-significant (0.092), suggesting that breeding for increased HC and plant biomass may be possible.

Adams et al. (2017b) examined these four high HC yielding Upland cotton (*G. hirsutum* L.) accessions (SA-1181, 1403, 1419, and 2269) grown both in field conditions and a greenhouse to compare the environmental effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves) under natural and controlled (protected) conditions. Leaf biomass was similar but higher in two field cultivated accessions. All four accessions produced higher % HC yields under field conditions, with greenhouse plants producing lower yields ranging from 20.6 to 46.0% as much HC as found in naturally field-grown plants. Total HC yields (g HC / g 10 leaves) were even lower in the greenhouse with yields being only 19.7 to 39.1% as high as from field-grown plants. Overall, the environmental component to the yield of free HC in cotton leaves was a major factor, with the genetic component contributing to less than half (46%) of the HC yield. This trend corresponds to literature reports of large induction of defense chemicals in cotton upon attack by herbivores and diseases (Chen 2008; Karban and Myers 1989; Opitz et al. 2008; Pare and Tumlinson 1997, 1998; Stipanovic et al. 1999; Turlings 1995). Ontogenetic variation in HC for SA-2269 showed HC yields in leaves remained at a constant, low level from bud to flowering, then increased rapidly as bolls matured (Adams et al. 2017b).

The purpose of this paper is to report on changes in HC production in four high HC yielding accessions of cotton (SA-1181, 1403, 1419, and 2269) grown with regular irrigation versus under limited irrigation or growing conditions to induce water stress similar to dryland stress field conditions in Lubbock, TX.

MATERIALS AND METHODS

Four accessions: (SA-1181Acala SJ-1,; 3010, SA-1403; China 86-1, SA-1419; TM 1, SA-2269) were grown at the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX under two irrigation regimes (regular and limited). Seed from each accession was planted at water field capacity and water was applied if needed until plant establishment. Irrigation regimes started after initial-squaring and continued through crop cutoff. Regular irrigation was set to 5mm/daily, and the limited irrigation or induced water stress similar to dryland stress conditions was set to 1.5 mm/daily. Each irrigation trial used a randomized complete block design with three replicated plots per accession. Irrigation was applied using sub-surface drip irrigation. Buffer or cotton plots were placed around and between each irrigation trial to ensure that one rate did not affect the plants within the other. In addition, irrigation rates were adjusted due to rainfall events to ensure differences in plant stress between the two irrigation rates, applying water only for one week for the limited irrigation trial for this experiment.

The ten (10) lowest growing mature leaves were collected from each of fifteen (15) plants for each of the four (4) accessions, in both regular irrigated and limited irrigation or dryland plots. At the time of collection, plants were at mid to completed full boll maturity, and leaves did not have insect or pest damage. Leaves were air-dried (48 hr., 45°C, to 7% moisture). Dried leaves were ground in a coffee mill (1mm). Three grams of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane. The jar was sealed and placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan, and the hexane was evaporated on a hot plate (70°C) in a hood. The pan with hydrocarbon extract was weighed and tared. The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. All shaker extraction yields were adjusted to oven dry wt (ODW) by a correction factor of 1.085. The soxhlet extraction correction factor was x1.14. Thus, the total correction factor (CF) utilized was 1.085 x 1.14 = 1.24.

Data were analyzed using proc glimmix with random block and block*accession, with adjust=simulate lines procedure for mean separation using the SAS statistical software (SAS Institute, Cary, NC, USA). In addition, statistical analyses (means, variance, standard deviation, standard error of mean, t-test) were performed by use of GrafPad.com and used for the figures and table reported herein. https://www.google.com/search?q=standard+deviation+calculator&oq=standard&aqs=chrome.2.69i57j69i60j35i39l2j0l2.3811j0j8&sourceid=chrome&ie=UTF-8

RESULTS

Analyses (using SAS) of interaction between GxE utilized pooled samples (all four accessions) revealed significant difference between irrigations and significant interaction between entries by irrigation or genotype by environment (GxE) for % HC yield and total HC ($p < 0.05$). Because of the GxE interaction, we separately analyzed the irrigation trials. When we examined the entries in each irrigation, no significant difference was found among entries for % yield HC, and significant difference at $p = 0.03$ was found for total HC under regular irrigation. When we examined the entries under limited irrigation or induced stress/dryland field conditions, significant difference at $p = 0.03$ were found for these two traits ($p < 0.001$). The average % HC yields and HC total were significant for SA-2269 compared to SA-1181, 1403, and 1419 under stress/dryland field conditions. In addition, high correlation was found between % HC yield and total HC ($r = 0.80$ regular and $r = 0.77$ limited irrigation) in the four accessions.

Comparison of the four accessions of cotton leaf biomass and HC yields for field grown under limited irrigation (from here on referred as dryland) and irrigated conditions, Lubbock, TX are given in Table 1. Note that none of the four accessions had significantly more biomass in irrigated than in dryland plots using the t test. This is because the summer of 2017 was unusually wet in Lubbock, leading to much smaller effects than normally experienced.

For detailed comparisons for each genotype, t-tests were run on each accession (15 plants under regular irrigation and 15 plants under reduced irrigation). The reader should note that the foregoing results differ a little from the above SAS analysis due to the different statistical models used. Using t-tests, the % HC yields were significant for SA-1181, and very highly significant for SA-1403, 1419 and 2269 (Table 1). The percent increase in HC yields (irrigated to dryland) were: SA-1181 18.1%; SA-1403 21.5%; SA-1419 30.3% and SA-2269 42.1%. The % HC yields were least affected by reduced irrigation in SA-1181, and the % HC was most increased by drought in SA-2269. Based on this factor alone (% HC yield), one would prefer SA-2269 if the crop were grown dryland in semi-arid lands.

Table 1. Comparison of four accessions of cotton leaf biomass and HC yields for field grown under dryland and irrigated conditions, Lubbock, TX. Significance: ns = non-significant; * = *p* 0.05; ** = *p* 0.01; *** = *p* 0.001.

	DW for 10 lvs/plant, +/- 2 std err.			% HC yield, +/- 2 std err.			HC g/ leaf DW, +/- 2 std err.		
Accession	dryland	irrigated	t-test	dryland	irrigated	t-test	dryland	irrigated	t-test
SA-1181	9.42 g +/-1.52	8.98 g +/-0.79	*p*= 0.253 ns	4.08 % +/- 0.30	3.34 % +/- 0.42	*p*= .0042 **	0.0381g +/-0.006	0.0305 g +/-0.005	*p*= 0.037 *
SA-1403	7.87 g +/-0.60	8.40 g +/-1.24	*p*= 0.225 ns	3.91 % +/-0.26	3.07 % +/-0.20	*p*= .0005 ***	0.0312g +/-0.003	0.0264 g +/-0.005	*p*= 0.053 ns
SA-1419	6.99 g +/-0.79	8.01 g +/-0.92	*p*= .05 *	4.19 % +/-0.84	2.92 % +/-0.39	*p*= .0005 ***	0.0293g +/-.004	0.0239 g +/-.004	*p*= 0.506 ns
SA-2269	8.99 g +/-0.59	9.63 g +/-0.63	*p*= 0.762 ns	5.35 % +/- 0.35	3.10 % +/- 0.30	*p*= .00005 ***	0.0487 g +/-0.005	0.0300 g +/-0.004	*p*= 0.0005 ***

The lack of variation in biomass (g DW 10 leaves) is shown in Figure 1. There is only one significant difference between dryland and irrigated in any of the accessions using the t-test.

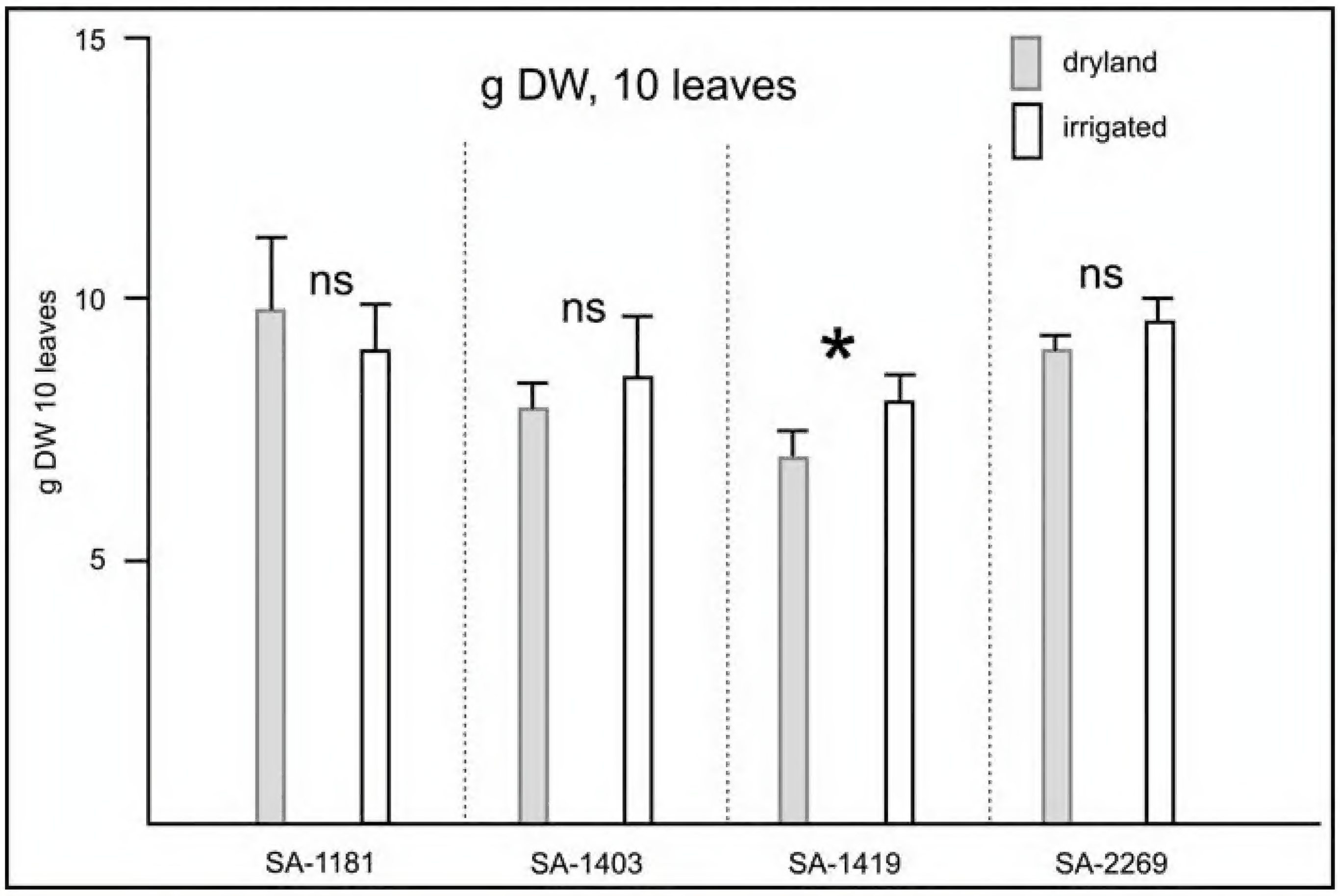

Figure 1. Bar graph of biomass (as g DW 10 leaves) for dryland and irrigated cotton accessions.

Figure 2 shows that each of the accessions had highly or very highly significantly larger % HC yields in dryland conditions. By far the largest increase was found with SA-1419 and SA-2269 having 30.3 and 42.1% higher % HC yields in dryland than in irrigated conditions (Fig. 2).

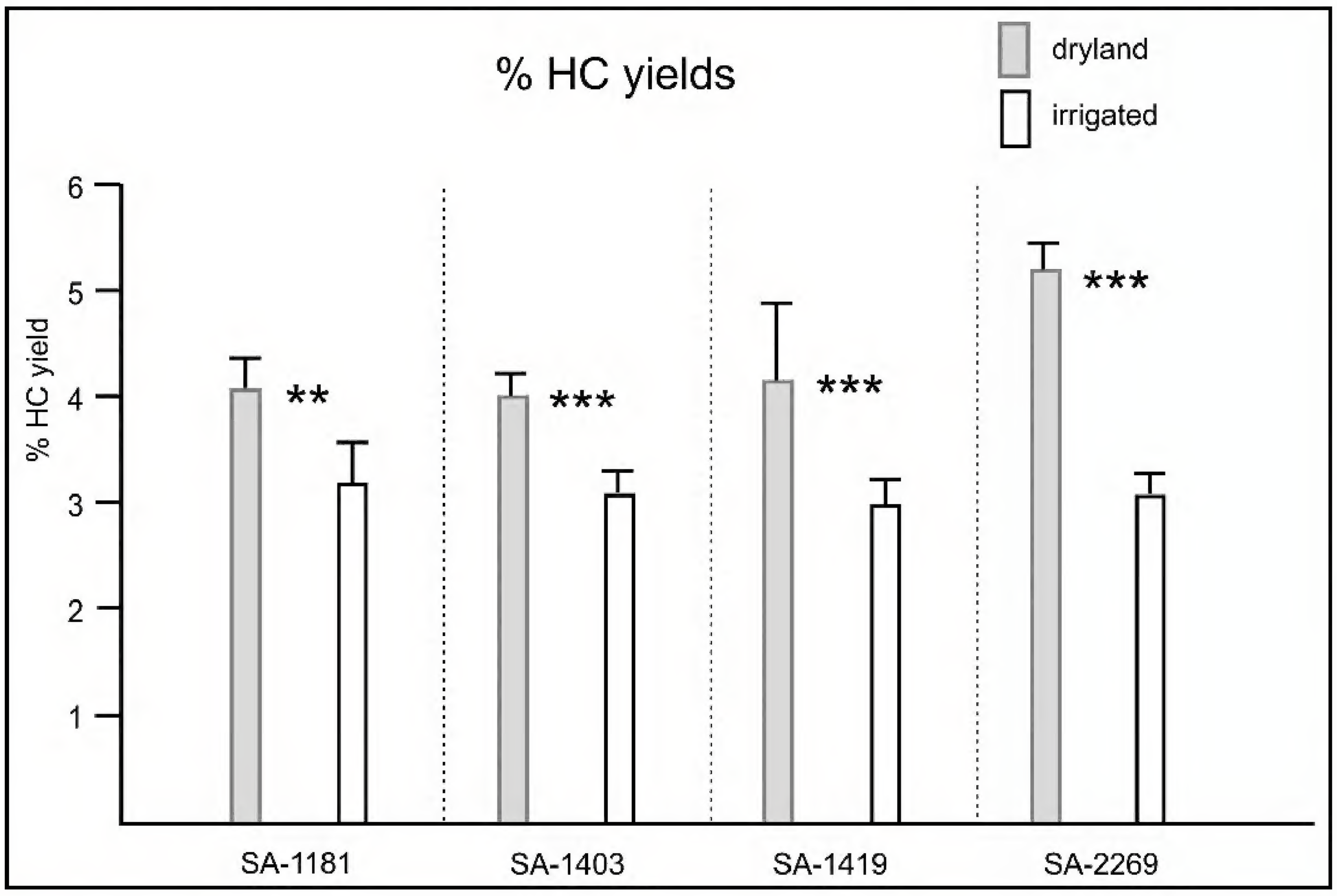

Figure 2. Bar graph of % HC yields for dryland and irrigated cotton accessions.

The HC yields (as g HC/leaf DW) were not significant for SA-1403 and SA-1419 under irrigated but with significant values for SA-1181 and highly significant differences under dryland conditions for SA-2269 due to the fact that the biomass was almost as large in dryland as in irrigated and % HC yield was also very much greater than in irrigated (Table 1.), thus, the product (biomass wt x % HC) was significantly greater (Table 1). Again, based on this factor alone (total g HC), one would prefer SA-2269 if the crop were grown dryland in semi-arid lands. Figure 3 shows all the accessions have larger HC yields (as g HC/ leaf DW), except SA-1403 and SA-1419 were not significant. Yields for SA-1181 and

SA-2269 were significant (*) and very highly significant (***). For SA-2269, the dryland g HC/ leaf DWyield was 38.4% greater than from irrigated plants.

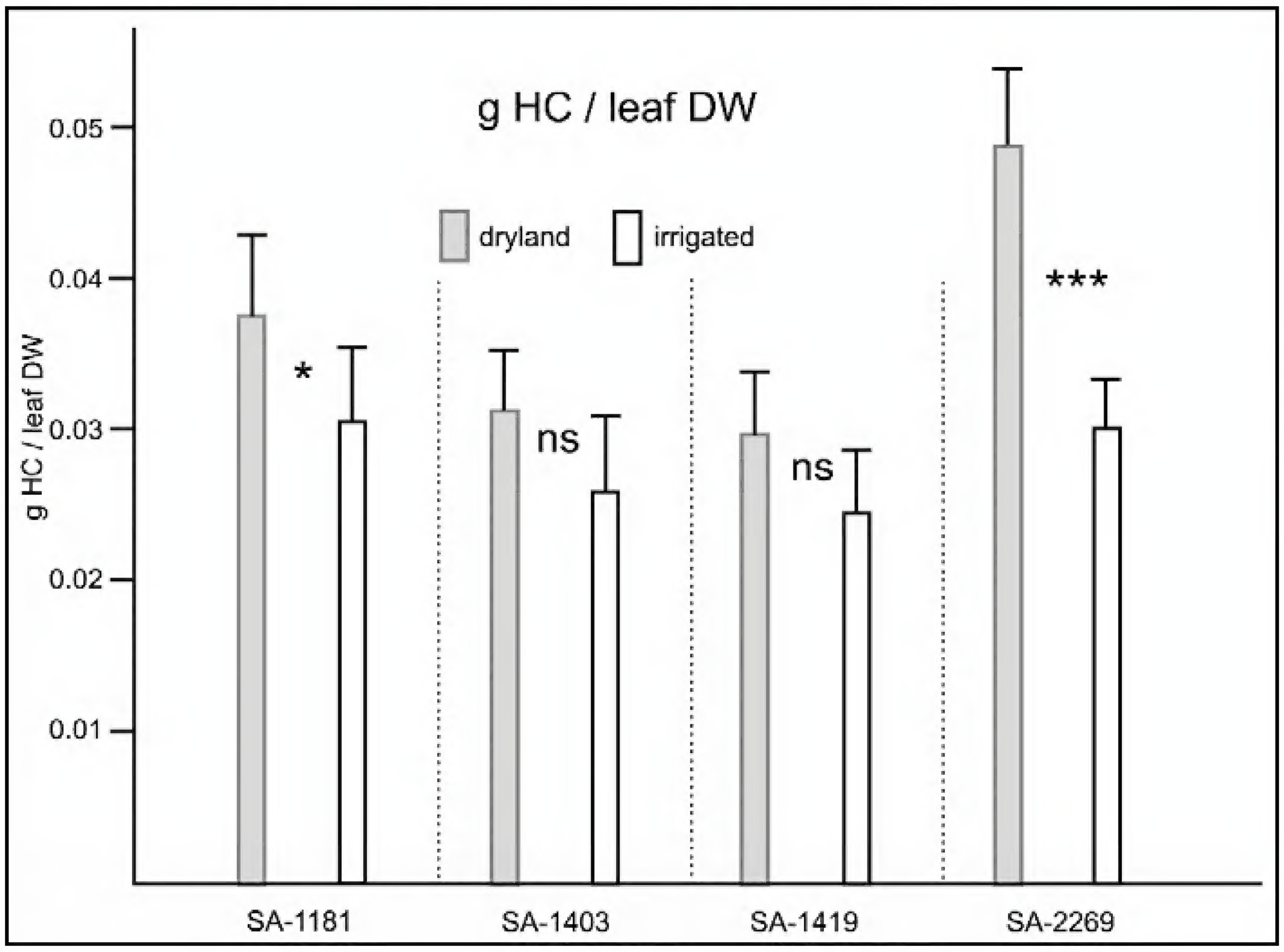

Figure 3. Bar graph of g HC/ leaf DW yields for dryland and irrigated cotton accessions.

Some of these % HC yield increases in dryland are actually moderate compared to the situation that occurred in College Station, TX, where, in the summer of 2016, these four accessions had much higher % HC yields: SA-1181 12.32%; SA-1403 9.08%; SA-1419 13.23% and SA-2269 11.09%. These larger amounts in the 2016 % HC yields appear to be due to some other factor than drought as they were grown on subsurface drip irrigation, in sandy soil, with an annual rainfall of 40" (Adams, et al. 2017a). It seems likely that these four accessions were damaged by insects or some disease and that led to the induction of defense chemicals, giving these atypically high yields in 2016 test plot.

The results from this study clearly show that the yields of HC are higher for these accessions when grown under water stress. In addition, there are some differential responses among the four accessions, with SA-2269 showing the greatest increase in HC yields in dryland conditions. Even though cotton is the most important renewable natural fiber, alternative sustainable, renewable sources of petrochemicals and fuels from arid and semi-arid land as the cotton crop may be another source of income for producers. In addition, in the Texas High Plains often-extended periods between rainfall events can lead to a reduction in the yield and fiber quality of cotton (*G. hirsutum*). Identifying morphological or physiological traits associated with plant water stress or drought could allow researchers or breeders to select/identify more efficiently for superior cotton with improved tolerance or sensitivity to drought. Further research (especially under normal, very dry summers in Lubbock) is needed to more fully understand these trends.

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Red floral nectar that absorbs ultraviolet light is produced by a new Peruvian species, *Jaltomata weigendiana* (Solanaceae)

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ABSTRACT

This is the first report of ultraviolet-absorbing corolla veins in the genus *Jaltomata*, and the first report of ultraviolet-absorbing nectar for the Solanaceae. The corolla of the species studied, *J. weigendiana,* is green and campanulate and reflects light in the ultraviolet (UV) part of the spectrum. The UV-absorbing radial corolla veins are hypothesized to serve as nectar guides. Five radial staminal-corolla thickenings create nectar troughs between the radial thickenings. The nectar is visibly red and, like the corolla veins, strongly absorbs UV radiation. Nectar volume from greenhouse-grown plants ranged from 7 to 57 µl (mean 28) and estimates of sugar concentration ranged from 9 to 24 % (mean 15). *J. weigendiana* is a new species, distinguished from congeners by having no more than two flowers per inflorescence and by lacking both a corolla limb and corolla lobules. Fruits of this species are eaten by local people. Published on-line www.phytologia.org *Phytologia 100(1): 12-18 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: colored nectar, edible fruit, foraging signal, Huánuco, nectar guide, Peru, protogyny, red nectar, UV absorptive nectar.

The genus *Jaltomata* includes about 70 diverse species, growing from Arizona, USA, to Bolivia, on the Galápagos Islands and in the Greater Antilles (Mione et al., 2015). Here we describe a new species having red floral nectar. The *Jaltomata* species having red floral nectar grow in the Andes of South America (Mione & Anderson, 1996; Hansen et al., 2007; Leiva González et al., 2015), and the new species we describe is from Department Huánuco, Peru. We collected this species during fieldwork in 2016.

MATERIALS AND METHODS

Plants were grown at Central Connecticut State University from seeds collected with the type collection. Ultraviolet photos were taken with a Canon EOS Rebel T5i camera having a Full Spectrum Conversion (www.lifepixel.com). To prevent visible and infrared light from entering the camera a StraightEdgeU (www.uvroptics.com) bandpass filter was used (on a Canon EF 50 mm f/1.8 lens with a Hoya +4 close-up). Flowers were illuminated with a UVP UVL-56 hand-held lamp. A UV-Visible Spectrophotometer (Evolution 201) was used to measure absorbance (3 µl of nectar were diluted to 20 ml in deionized water). Nectar volume was measured with microcapillary tubes and sugar concentration was estimated with a refractometer. Six flowers of unknown age (fully open but not senescing) were used; nectar had not been removed previously.

Stalked glands have multicellular heads and unicellular stalks, the heads staining densely with neutral red (as illustrated in Mione & Serazo, 1999). The other hairs lack multicellular heads: dendritic hairs are uniseriate and many-celled with three or more branches emerging at different levels; forked hairs are also uniseriate and many-celled but have only two termini; finger hairs are uniseriate and unbranched. In the following description, unless indicated otherwise, hairs are not gland-tipped and are unpigmented. Pollen size is based on 28 grains in polar view stained with cotton blue in lactoglycerol. The reported seed counts and fruit size are from two field-collected fruits. Species authorities are provided in Appendix 1.

Jaltomata weigendiana Mione & S. Leiva sp. nov. Type: Peru. Dept. Huánuco: Prov. Huamalíes, 3,727 m, 9 24' 54" S, 76 45' 24.6" W, rock wall between agricultural fields, 21 & 22 May 2016, *T. Mione, S. Leiva G. & L. Yacher 873* (F). (Figures 1 & 2).

Inflorescence 1–2 flowered, corolla campanulate and green, lacking a corona, corolla limb and corolla lobules, radial staminal-corolla thickenings (five) creating nectar troughs (five) between the radial thickenings, the stigma capitate.

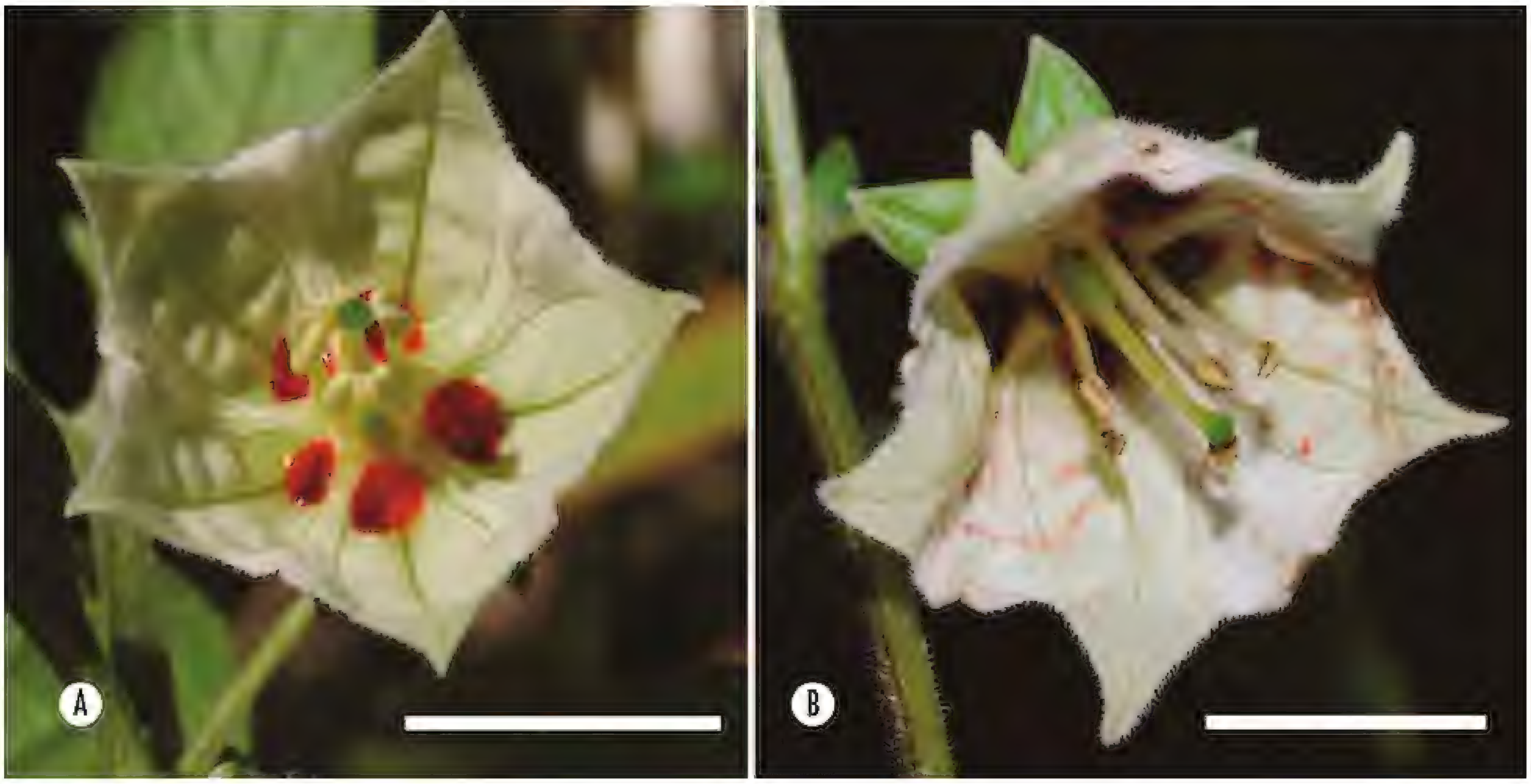

Figure 1. Protogyny of *Jaltomata weigendiana.* **A**. Pistillate phase (anthers undehisced). **B**. Hermaphroditic phase (anthers dehisced). Scale bars represent 1 cm. **A**. Cultivated plant, in sunlight, by Sara K. Popolizio. **B**. At the site of the type collection, with a flash, by T. M.

Shrub 1–1.5 m high, the young branches green, pubescent with erect, finger, forked and dendritic hairs, sometimes gland tipped**,** the older stems brown, glabrous, with lenticels (Figure 2E)**,** terete**,** and to 1.2 cm diameter. Leaves alternate or geminate, the blade to 10 cm long × 5.6 cm wide but commonly smaller, darker green above, ovate to elliptical-lanceolate**,** the apex acute (Figure 2C)**,** both faces densely pubescent with finger, forked and dendritic hairs**;** petiole to 2.2 cm long**.** Inflorescence 1–2 flowered; peduncle 4–7 mm long, green at flowering, terete, pubescent with erect, finger, forked and dendritic hairs; pedicel 10–11 mm long**,** green at flowering, terete**,** pubescent with erect, finger, forked and dendritic hairs**.** Calyx green, 15–20 mm diameter**,** the lobes weakly recurved (Figures 1B, 2A)**,** adaxially with a dense covering of stalked glands (that have multicellular heads) 0.06 mm long**,** abaxially pubescent with dendritic, forked and finger hairs; the calyx 23.5–30 mm diameter at fruit maturity (Figure 2D). Corolla green, with radial UV absorbing veins (Figure 2A), campanulate, 8–10 long × 25–30 mm diameter, 5-lobed and lacking lobules (Figure 1), externally pubescent the hairs 0.2 to 0.25 mm long, mostly fingerlike, but some forked and a few dendritic, internally puberulent with gland-tipped finger hairs 0.1–0.3 mm long; the nectar showing through the wall of the corolla. Stamens 9–10.5 mm long, exserted 3 mm beyond distal end of corolla; the radially oriented staminal-corolla thickenings create troughs between the radial thickenings, with copious red nectar pooling in troughs (Figure 1); the base of the filament expanded laterally (ventral view), the filaments connivent prior to anther dehiscence (Figure 1A), angling outward after anther dehiscence (Figure 1B); pale-green to off-white, pilose along 24–33% of the length proximally, with unpigmented finger hairs; the anther yellow (Figure 1A) with a green connective prior to dehiscence, 1.7 (dehisced)–2.8 (undehisced) long × 1.7 mm wide (fresh), not mucronate; mean pollen grain diameter 34 µm. Stigma capitate, 0.8–0.85 × 0.95–1.05 mm, with a shallow medial groove, darker green than the style (Figure 1); style 8.2–8.5 mm long, straight, pale-green, exserted a few mm beyond distal end of corolla; ovary green, 2.8 high × 2.6 mm wide, the nectary disk orange and half as high as the ovary. Fruit orange (Figure 2B,C), 1.5 × 2 cm; 332–362 seeds per fruit.

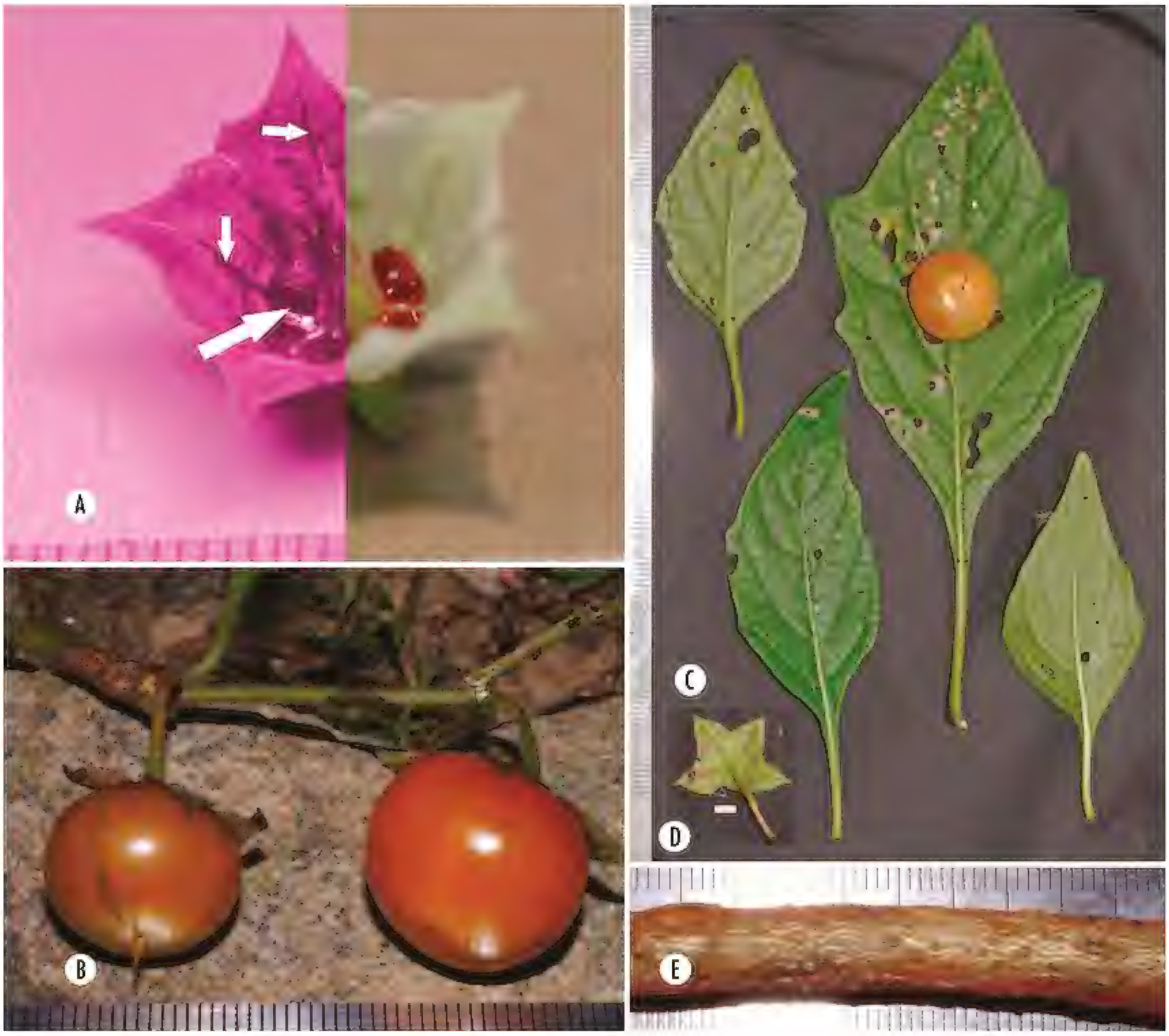

Figure 2. *Jaltomata weigendiana.* **A**. Two photos of the same flower joined together: UV only on left, smaller arrows–UV absorptive nectar guide, larger arrow–nectar; visible light on right; units are mm. **B**. Fruits, orange when ripe; units are mm. **C**. Leaves and a ripe fruit, lower right leaf is lower face up; units along left are mm. **D**. Calyx from ripe fruit, one lobe missing due to herbivore damage; segment of ruler = 5 mm. **E**. Older branch with lenticels; units are mm. All photos were taken at the site of the type collection except **A**. was a cultivated plant; all photos were taken by T. M.

Additional specimens examined. PERU**.** Huánuco**:** Prov. Huamalíes, 9° 26' S, 76° 46' W, above Chavin De Pariarca, hedges between fields, 3600 m, 19 Mar 2001, *M. Weigend, K. Weigend, M. Binder & E. Rodriguez 5246* (Ref 2001/246, 6) (M); 3,727 m, 9 24' 54 S, 76 45' 24.6" W, rock wall between agricultural fields, 21/ 22 May 2016, *S. Leiva G. et al. 6065* (HAO), *Mione et al. 874* (CCSU).

Table I. Comparison of *Jaltomata weigendiana* with similar congeneric species.

	J. weigendiana	*J. herrerae*	*J. estilopilosa*	*J. paneroi*	*J. neei*	*J. alviteziana*	*J. dendroidea*	*J. leivae*
Country, Primary Political	Peru, Huánuco	Peru, Cuzco, Apurimac, Ayacucho. Bolivia, La Paz	Peru, Amazonas	Peru, Cajamarca	Peru, Cajamarca	Peru, Cajamarca	Peru, La Libertad	Peru, Cajamarca
Altitude (m)	3,600– 3,727	3000 – 3750	2994	3240 – 3500	2400 - 2919	2960–3084	3100 – 3360	2560–2650
Leaf blade	pubescent	pubescent	pubescent	pubescent	pubescent	pubescent	pubescent	glabrate
Flowers Per Inflorescence	1–2	2–6	2–4	3–6	1–5	2–3	1–4	1–3
Calyx Diameter, flowering (mm)	14–19	21–26	11–15	13	16–18	13–20	17–21	15
Calyx Lobes	as long as wide	longer than wide	as long as wide	as long as wide	as long as wide	as long as wide	as long as wide	as long as wide
Calyx reflexed during anthesis	weakly	strongly	weakly	no	no	weakly to strongly	no to weakly	strongly
Calyx diameter, ripe fruit (mm)	30	40	20	25	25	20–28	23–25	26
Corolla color	green	green	green	pale-green	green turning blue	purple-blue	green	purple-blue
Purple pigment in base of corolla	no	yes	no data	yes	yes	yes	yes	yes
Corolla shape	campanulate	campanulate	tubular-campanulate	tubular-campanulate	tubular-campanulate	tubular-campanulate	tubular-campanulate	tubular-campanulate
Corolla Limb	no	yes	yes	yes	yes	yes	yes	yes
Corolla lobes / lobules	5 / 0	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5	5 / 5*	5 / 5
Corolla Diameter	25–30	30–40	24–25	30–35	25–39	25–27	23–30	14
Corolla Length (mm)	8–10	10–15	10–11	5–10	9–13	8–12	10–13	7–9
Percent of length of filament pubescent, proximal to distal	24–33	3–5	3–5	3–15	5–10	2–3	to 23	20–25
Style length	8.2–8.5	14	11–11.5	7–11	11–13	7–10.2	12–12.5	9–18

* The corolla lobules of *J. dendroidea* were absent on one collection (*Mione et al. 722*) in the wild, but were present at the type collection and were present on flowers of plants of *Mione et al. 719* grown by Jamie Kostyun.

Jaltomata weigendiana grows in Peru, Department Huánuco, province Huamalíes, between 3,600 and 3,727 m in the vegetation between agricultural fields. No other species of the genus *Jaltomata* has only one to two flowers per inflorescence, red nectar, and lacks a corona, a corolla limb and corolla lobules. Table 1 compares *J. weigendiana* with similar congeneric species: shrubs having a campanulate, tubular-campanulate or tubular-urceolate corolla, copious red/orange nectar, radial staminal-corolla thickenings creating five nectar troughs (one trough between each pair of thickenings), no corona, stamens connivent prior to anther dehiscence, yellow anthers but with a green connective on the dorsal face, and a capitate stigma. The corolla of *J. dendroidea, J. paneroi and J. weigendiana* remain open at night, and the corolla of other species in Table 1 have not been studied but almost certainly remain open at night given their similar corolla form.

We estimated the population size to be less than 1000 mature individuals, and thus using criterion D1 of the IUCN (2017) *J. weigendiana* is considered vulnerable (VU). Collections of this species were not present among the specimens borrowed from various herbaria, and thus it seems that the only place where it has been collected is the type locality. From discussion with two adults who live near the type locality we learned that the ripe berries are eaten by locals, "se comen," and the local name of this species is "antaraura." The name of this species was chosen to honor Dr. Maximilian Weigend who was the first to collect this species.

Flowers of *J. weigendiana* are protogynous with the pistillate phase (anthers undehisced, Figure 1A) followed by the hermaphroditic phase (anthers dehisced, Figure 1B). The flowers remain open at night; in contrast flowers of congeners having rotate corollas close at night (Mione, 1992). Although self-compatible, flowers of *J. weigendiana* are not autogamous. On plants grown for study nearly no fruit was set without manual pollination, and after manual self-pollination fruits developed and ripened in seven weeks.

In the visible spectrum the nectar of *J. weigendiana* is orange when flowers first open and subsequently turns red with age. The darkening of nectar within flowers as they age has been reported in the Lamiaceae (Zhang et al., 2012) and in *J. calliantha* (Plourd & Mione, 2016). The nectar of *J. weigendiana* absorbs ultraviolet, violet, blue and green wavelengths (Figure 3). Colored nectar that is UV-absorptive has previously been reported (Zhang et al., 2012). However, this is the first report of colored, UV-absorptive nectar for the Solanaceae. While taking into consideration that UV light is not more obvious to pollinators than other bands that nonhuman animals see (Kevan et al., 2001), it is possible that the contrast between the relatively UV-reflective corolla and the UV-absorptive nectar and nectar guides (Figure 2A) enhances the foraging signal (Thorp et al., 1975; Frohlich, 1976). Nectar is

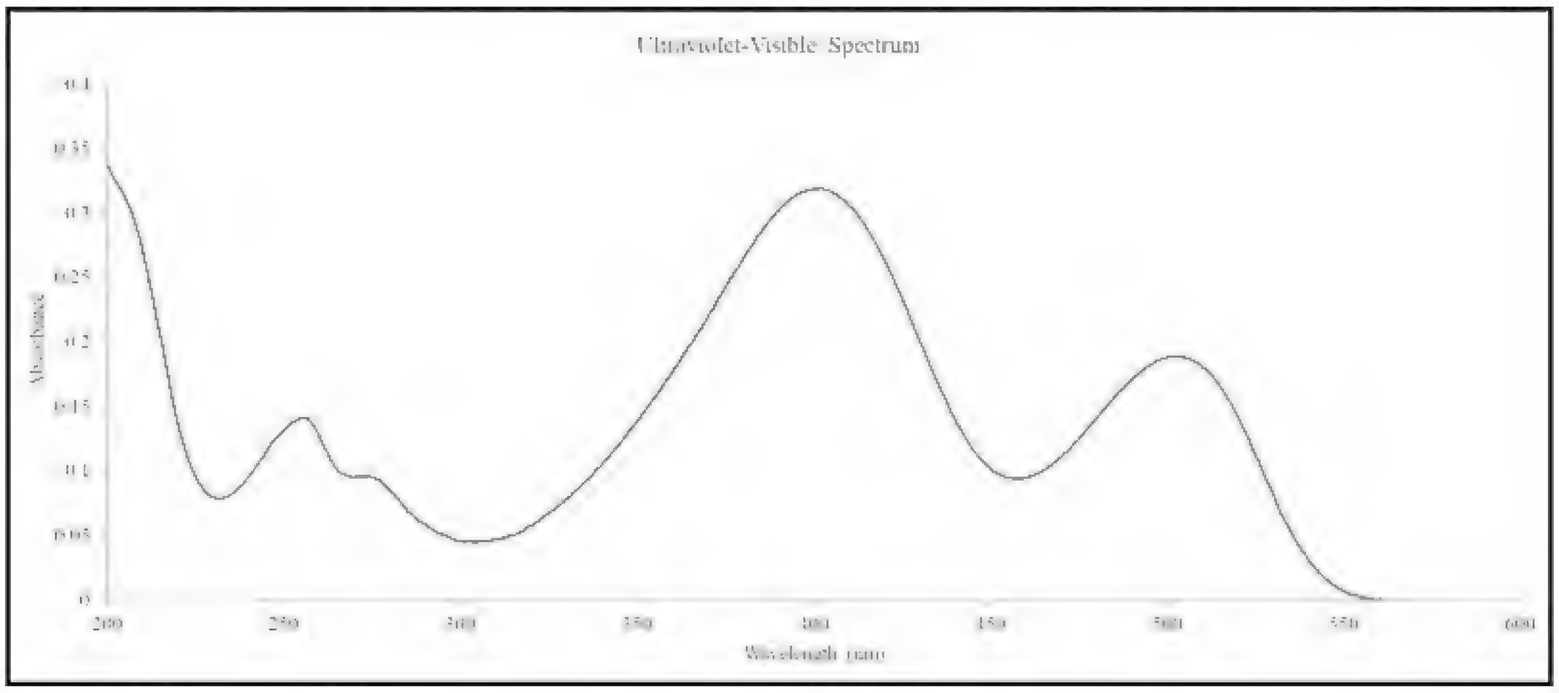

Figure 3. Spectrophotometric analysis of floral nectar from the flower of a cultivated plant of *Jaltomata weigendiana*.

produced by flowers in both the pistillate (Figure 1A) and hermaphroditic phases (Figure 1B). *J. quipuscoae* has a nectar absorbance spectrum (Mione, unpublished) that is nearly identical to that shown for *J. weigendiana* (Figure 3). These two species are the first to have been examined for UV absorbance; it seems likely that most or all of the *Jaltomata* species having red nectar (illustrated and shown in photographs by Leiva et al., 2015) will show a similar absorbance spectrum.

At least some insects (Chittka et al., 1994), bats (Winter et al., 2003), and hummingbirds (Goldsmith, 1980; Jacobs, 1992; Tovée, 1995) see ultraviolet wavelengths. Although we have not observed pollinators visiting *J. weigendiana*, the pollinators of a few other *Jaltomata* species have been recorded. Bees have been seen visiting *Jaltomata* spp. that have rotate or short-tubular corollas, all lacking red nectar (*J. procumbens* and *J. repandidentata* in Mexico and Costa Rica [Eickwort, 1967; Williams, 1985], and *J. athahuallpae and J. diversa* in Peru [Mione & Leiva G., unpublished]). Hummingbirds have been photographed visiting the long-tubular flowers (lacking red nectar) of *J. viridiflora* in Ecuador and the campanulate flowers (having red nectar) of *J. calliantha* in Peru (Leiva G. & Mione, unpublished). The corolla of *J. weigendiana* is campanulate and green with red nectar, and the red coloration and UV contrast (including that created by the nectar guides) may be foraging signals for hummingbirds. The red coloration may also attract insect pollinators given that there are insects with "specialised red receptors" and red is "not invisible to insects that lack a specific red receptor," (Kevan et al., 2001, p. 2577). In flowers of plants of *J. weigendiana* grown for study, nectar volume ranged from 7 to 57 µl (mean 28) and estimates of sugar concentration ranged from 9 to 24 % (mean 15). These sugar concentrations are similar to those reported for flowers visited by hummingbirds, hawkmoths, butterflies and bats (Pyke & Waser, 1981).

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Appendix 1. *Jaltomata* species, with authorities, mentioned in the text and Table 1.

J. alviteziana S. Leiva	*J. estilopilosa* S. Leiva & Mione	*J. procumbens* (Cav.) J. L. Gentry
J. athahuallpae S. Leiva & Mione	*J. herrerae* (C. V. Morton) Mione	*J. quipuscoae* Mione & S. Leiva
J. calliantha S. Leiva & Mione	*J. leivae* Mione	*J. repandidentata* (Dunal) Hunz.
J. dendroidea S. Leiva & Mione	*J. neei* Mione & S. Leiva	*J. viridiflora* (Humb., Bonpl. & Kunth) M. Nee & Mione
J. diversa (J. F. Macbr.) Mione	*J. paneoi* Mione & S. Leiva	*J. weigendiana* Mione & S. Leiva

The *Juniperus flaccida-J. poblana* complex revisited: insights from molecular and oil analysis

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ABSTRACT

Analysis of nrDNA, petN-psbM, trnS-trnG, trnD-trnT, and trnF-trnL revealed that *J. flaccida* is diverse, but quite separated from *J. poblana* and *J.* aff. *poblana* from Nayarit by at least 10 Mutational events (MEs, SNPs + indels), and is actually a bit closer to *J. standleyi* (8 MEs). A divergent *J. poblana* from Oaxaca is merely 2 MEs distant from the Nayarit trees. The *J. poblana* samples from the type locality (Amozoc, Pue.) vary by only 1 ME among samples. Leaf oils, on the other hand, show to the Nayarit juniper quite differentiated from other *J. poblana*. In addition, the habit (large trees with a strong central axis with branchlets long, pendulous and very planate) and habitat of the Nayarit trees support the recognition of it as a different taxon closely related to *J. poblana*. However, additional evidence is needed in order to resolve its taxonomic status. Published on-line www.phytologia.org *Phytologia 100(1): 19-26 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: *J. flaccida, J. poblana*, Cupressaceae, Nayarit, nrDNA, petN-psbM DNA, terpenes.

Juniperus flaccida Schltdl. and *J. poblana* (Martínez) R.P. Adams are closely related species with large, multi-seeded cones and weeping (flaccid) foliage that make them difficult to differientiate morphologically (Adams 2014). Recently, we reported (Adams et al. 2017a) on the discovery of large, beautiful trees in a new population of putative *Juniperus poblana* from Nayarit, Mexico. The trees have a very strong central axis and long, pendulous foliage. They are magnificent on the rocky areas where they occur.

Recently, analysis of DNA sequences (Adams et al. 2017a), placed those Nayarit trees in a clade with *J. poblana*, but the Nayarit trees were in a well-supported sub-clade. However, samples of *J. poblana* exhibited considerable variation and, because no samples were included from Amozoc (the type locality), it seemed premature to make a decision about the taxonomic status of the Nayarit trees.

Additional samples were collected of *J. flaccida* from the Chihuahuan desert and *J. poblana*, from the type locality, Amozoc. The volatile leaf oils of the taxa were analyzed and revealed (Adams et al. 2017b) that the oils from the Nayarit trees were quite distinct (Fig. 1). Notice that the first coordinate (34%) largely separates the Nayarit trees from all the other OTUs (Fig. 1). This finding was surprising, as the DNA sequencing placed the Nayarit trees in an unresolved polytomy with *J. poblana* (Adams et al. 2017a).

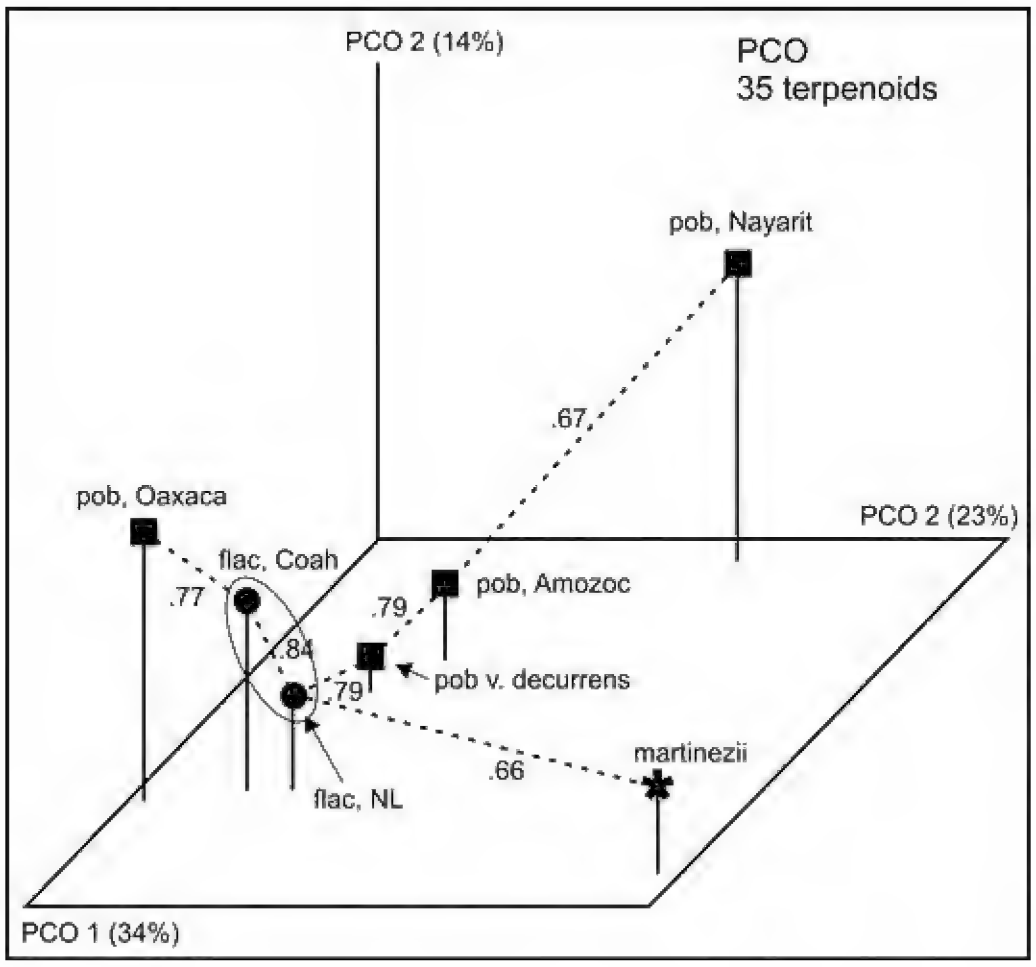

Figure 1. PCO based on 35 terpenoids from the volatile leaf oils of *J. poblana*, *J. flaccida*, *J. martinezii*, and the Nayarit trees. The dotted lines are the minimum spanning network. The numbers next to the dotted lines are the similarities (1 to 0.0). NL = Nuevo Leon. Coah = Coahuila.

The purpose of this study was to further explore the position of the magnificent Nayarit trees of putative *J. poblana* through DNA sequencing of additional samples of *J. flaccida* and *J. poblana.*

MATERIALS AND METHODS

Plant material and populations studied:

J. flaccida, Short trees, 1.5-3 m tall; bark on branches papery and exfoliating, inner bark smooth, reddish; no seed cones, similar to *J. flaccida,* but in a very dry habitat in the Chihuahuan desert region, Mexico, Durango, Mpio. Lerdo, Sierra del Rosario, nearly atop the mountain, with *Yucca* and oak scrub; on limestone, 25° 38’ 44” N, 103° 54’ 40” W, 2700 m, 8 Apr 2008, Coll. *M. S. Gonzalez-Elizondo et al. 7375 a,b;* Lab Acc. *Robert P. Adams 14616, 14617.*

J. aff. *poblana,* uncommon young trees (saplings) 2 m, in oak woodland dominated by *Quercus resinosa*, Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz, Km 86.8; S of bridge of arroyo del Fraile, E of El Maguey, 22° 10’ 08” N, 104° 43’ 51” W, 1150 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8381 with L. López, A. Torres Soto;* Lab Acc. *Robert P. Adams 14896*

J. aff. *poblana,* large, single stemmed trees, foliage long and pendulous, abundant trees, up to 25 m high, on strongly rocky slope, forest of *Juniperus-Clusia* with elements of mesophytic forest (Magnolia) and tropical forest (*Bursera*, *Opuntia*, *Pilosocereus purpusii*) as well as *Agave attenuata* and *Yucca jaliscensis,* Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz; NE of El Maguey, 22° 07’40” N, 104° 47’ 47” W, 1430 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8379a,b,c,d, with L. López, A. Torres Soto;* Lab Acc. *Robert P. Adams14897-14900,*

J. flaccida, Adams 6892-6896, 23 km E. of San Roberto Junction on Mex. 60, Nuevo Leon, Mexico;

J. flaccida, Reserva Ecologica Municipal de Sierra y Cañon de Jimulco, 25° 07' 38" N, 103° 16' 15" W., 2118 m, 17 Jan 2017, Torreon, Coahuila, Mexico, Coll. *Manuel Rodríguez Munoz et al. #1,2,3,4,5,* Lab Acc. *Adams 15203 - 15207*;

J. martinezii, *Adams 5950-5952, 8709*, 40 km n of Lagos de Moreno on Mex. 85 to Amarillo, thence 10 km E. to La Quebrada Ranch, 21° 33.08' N, 101° 32.57' W, Jalisco, Mexico;

J. poblana var. *decurrens, R. P. Adams 11926, 11927, 11928,* small trees, to 5 m tall, with strong central axis, foliage very, very, weeping, common, about 2 km S. of Valle de Topia. All leaves decurrent, and prickly and are not merely juvenile leaves. 25° 14' 11" N; 106° 26' 55.7" W, 1818 m, 30 Jun 2009, Durango, Mexico;

J. poblana, *Adams 6868-6870*, 62 km S. of Oaxaca, Mexico on Mex. 190.

Voucher specimens are deposited at BAYLU and CIIDIR when applicable.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

Sequencing nrDNA, petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF resulted in 4,351 bp of concatenated sequence data. A Bayesian tree shows (Fig. 2) *J. poblana* (Oaxaca), *J. poblana* var. *decurrens* (Adams and Schwarzbach, 2015) (red shaded), *J. flaccida*, (yellow) and *J. poblana* (Amozoc, type locality) and the Nayarit junipers (blue) are all grouped in one clade with high support.

Within this clade, three sub-clades are supported: *J. poblana* (Oaxaca), *J. poblana* var. *decurrens* (red shaded), *J. flaccida*, (yellow) and *J. poblana* (Amozoc, type lcn.) and the Nayarit junipers (blue) (Fig. 2). Notice that all the Nayarit junipers have little or no variation in their DNA sequences (Fig. 2). In addition, two samples of *J. poblana*, Amozoc, show few or no differences from the Nayarit junipers (Fig. 2).

To visualize the variation in molecular events (MEs = SNPs + indels) among individuals, a minimum spanning network (MSN) was constructed using both nucleotide substitutions and indel data. Whereas, the Bayesian tree is a flattened 2-demensional representation, the MSN has more of a 3-dimensional perspective. In addition, one can see exactly the magnitude of differences separating the groups. A few related, but taxonomically distinct species are included in the MSN. Notice that *J. standleyi, J. monticola, J. jaliscana, J. durangensis* and *J. martinezii* are each separated from adjacent taxa by 6 to 11 MEs (Fig. 2). This metric (6-11) is useful to keep in mind, when we consider specific levels among *J. flaccida*, *J. poblana* and the Nayarit junipers.

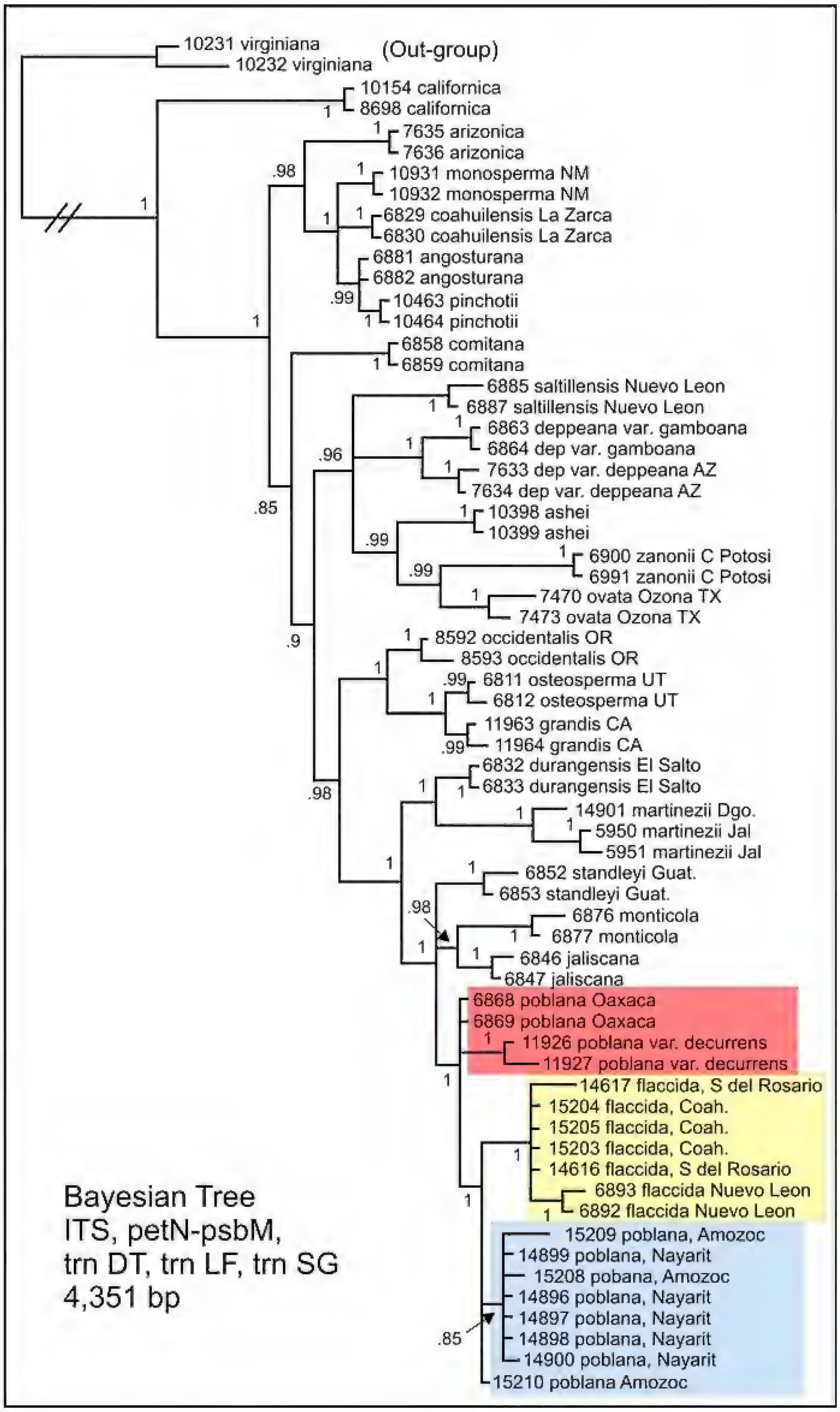

Figure 2. Bayesian tree based on sequences from ITS (nrDNA), petN-psbM, trn D-trn T, trn L- trn F, and trn S -trn G. Numbers at the branch points are posterior probabilities. *J. poblana*, Oaxaca, and *J. poblana* var. *decurrens* are in red shading. *J. flaccida* individuals are in yellow, and *J. poblana*, Nayarit and Amozoc are in blue shading. See text for discussion.

The MSN shows *J. flaccida* is diverse, but quite separated from *J. poblana* and the Nayarit junipers by at least 10 MEs, but actually a bit closer to *J. standleyi* (8 MEs, Fig. 3). No variation is seen among the five (5) Nayarit junipers in their DNA sequences (Fig. 2). The 'divergent' *J. poblana* from Oaxaca (as per the Bayesian tree, Fig. 1), is merely 2 MEs distant from the Nayarit trees. The *J. poblana* samples from the type locality (Amozoc, Pue.) vary by only 1 ME among samples (Fig. 3). The *J. poblana* var. *decurrens* samples are separated from *J. poblana* by 4 MEs (Fig. 3). This divergence of 4 MEs is about half of the amount (6-11 MEs) typically found between species (in this study), and thus, the DNA divergence supports the recognition of *J. poblana* var. *decurrens* as a variety.

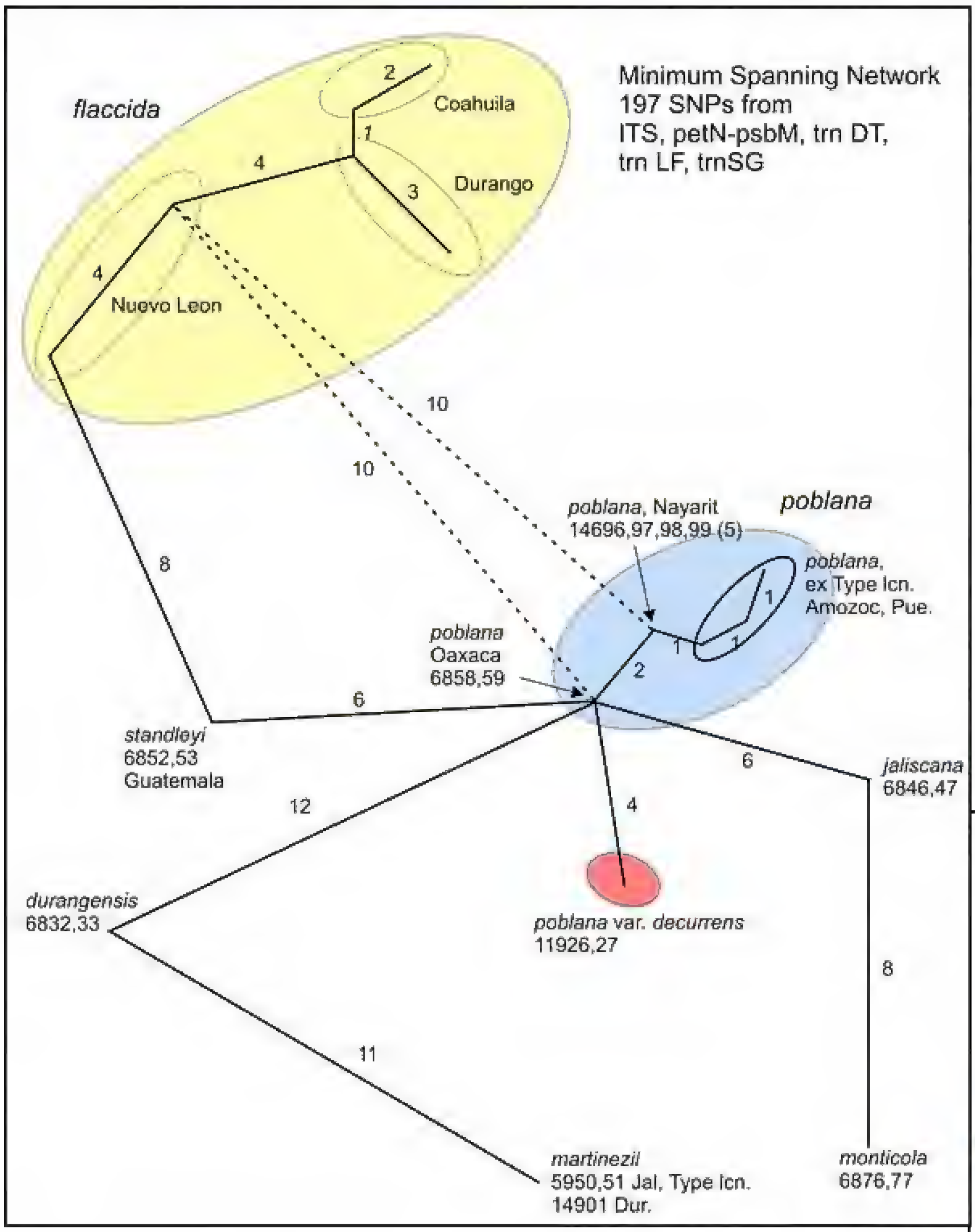

Figure 3. Minimum spanning network based on SNPs and indels in ITS (nrDNA), petN-psbM, trn D-trn T, trn L- trn F, and trn S -trn G. The numbers next to the lines are the number of MEs (mutational events = SNPs + indels). The two dashed lines are the 2nd shortest links between *J. flaccida* and *J. poblana.*

It is interesting to examine a minimum spanning network based on the leaf terpenoids (Fig. 4). Notice that the leaf oil from the Nayarit juniper is quite divergent from *J. poblana*, Amozoc oil (Fig. 4). So, in contrast to the ITS and cp DNA, the oil data shows the Nayarit junipers to have some genetic differentiation.

In addition, differentiation is seen (Fig. 4) within *J. poblana* as the oil of *J. poblana* (Oaxaca) is more similar to the oil of *J. flaccida* than other the oils of other *J. poblana.*

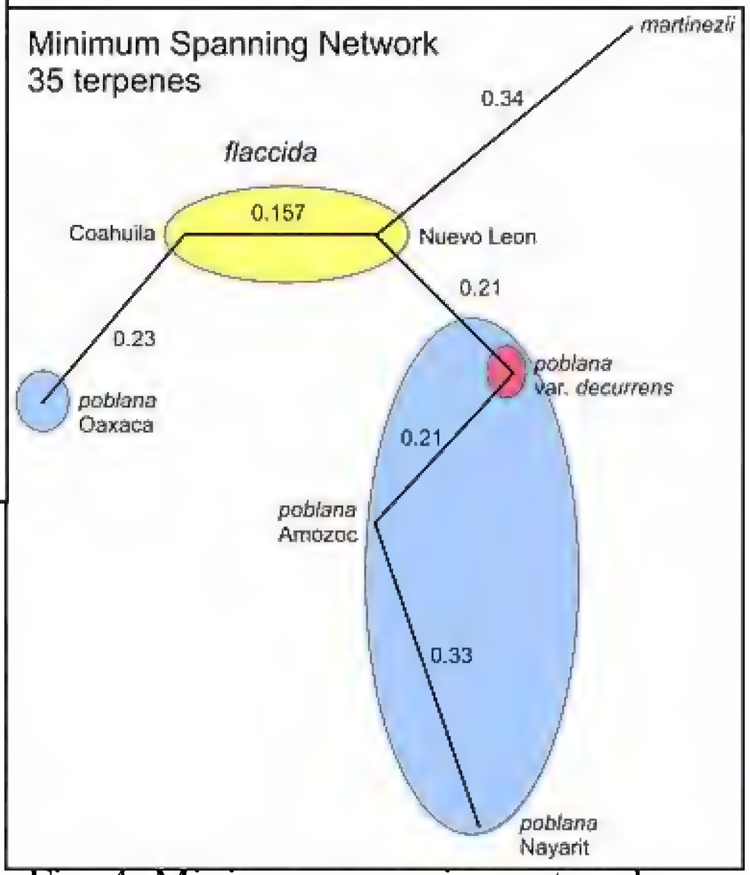

Fig. 4. Minimum spanning network based on 35 leaf terpenoids. The numbers next to the lines are distances scaled as: [1.0 - terpene similarity].

The Nayarit junipers are clearly differentiated in their habit (Fig. 5), having a strong central axis and long trunk vs. *J. poblana* south of Oaxaca (Figs. 6, 7) and *J. p.* var. *decurrens* (Fig. 8).

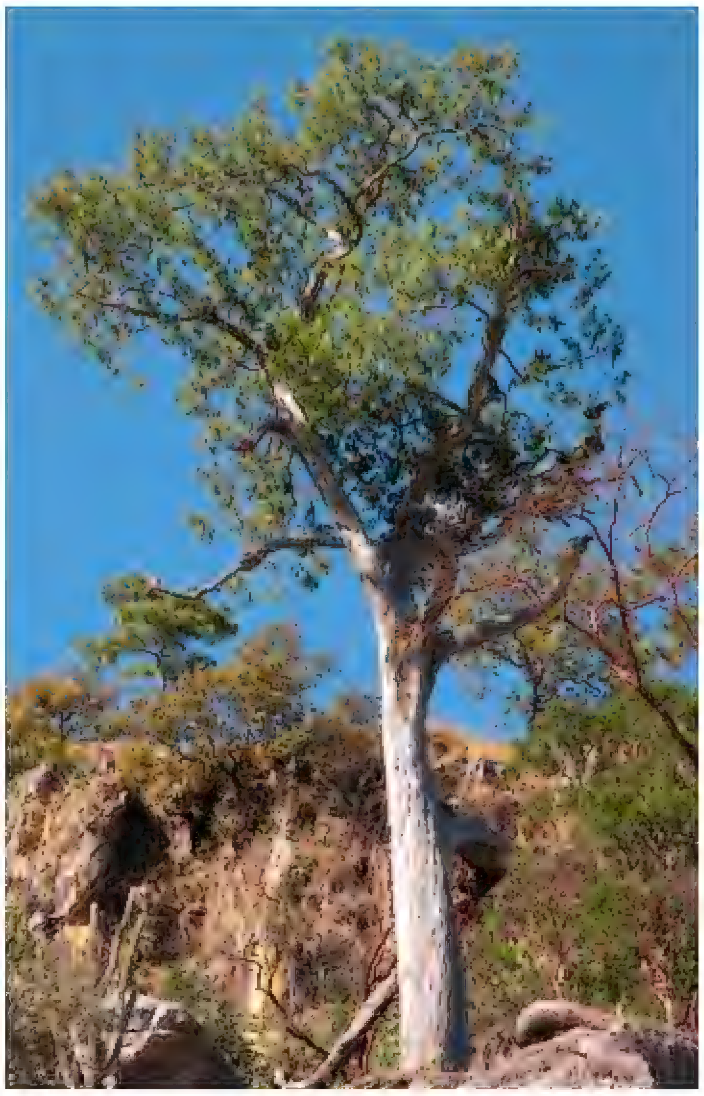

Fig. 5. Nayarit juniper as large tree with a strong central axis.

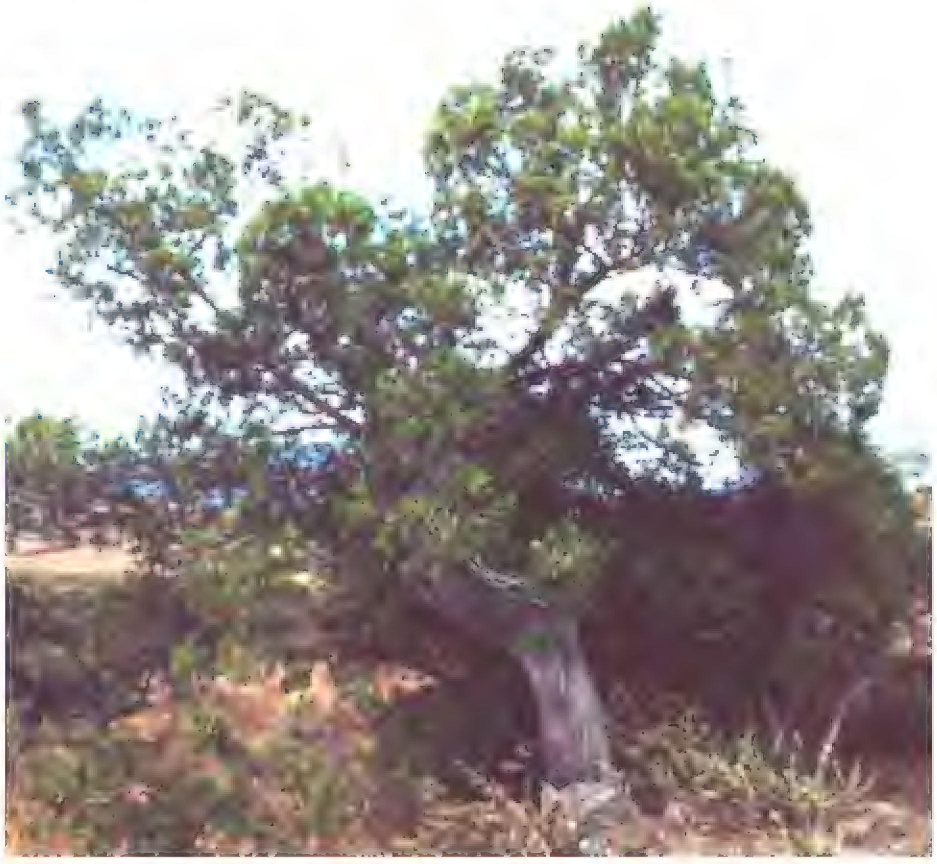

Fig. 6. *J. poblana,* habit, at KM 62 S of Oaxaca.

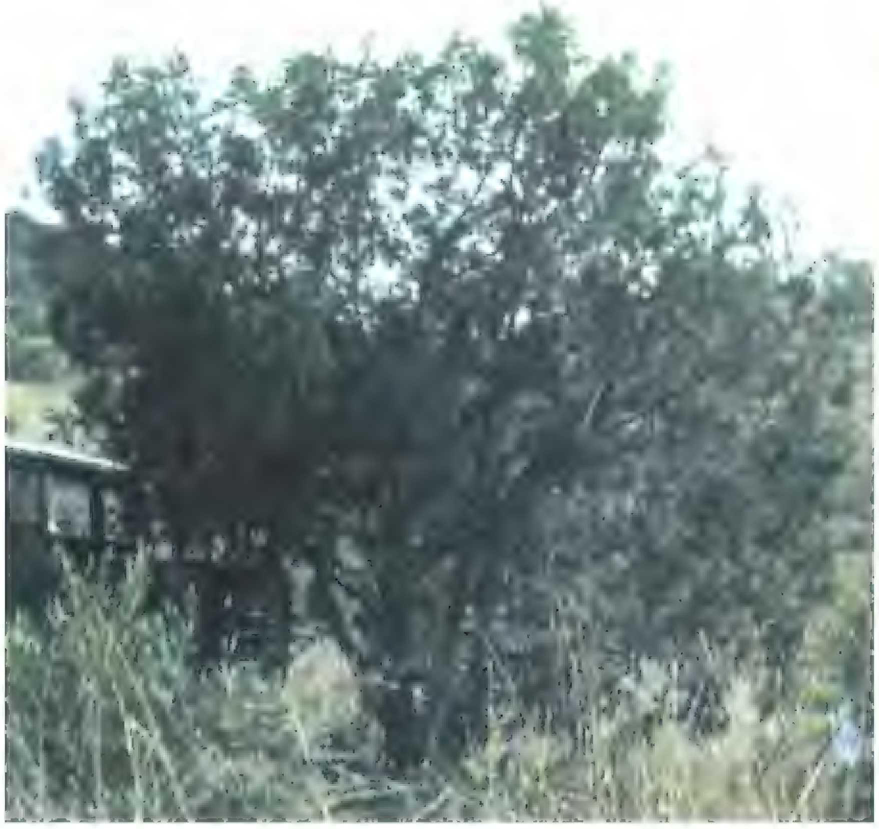

Fig. 7. *J. poblana*, habit, at Km 62, S of Oaxaca.

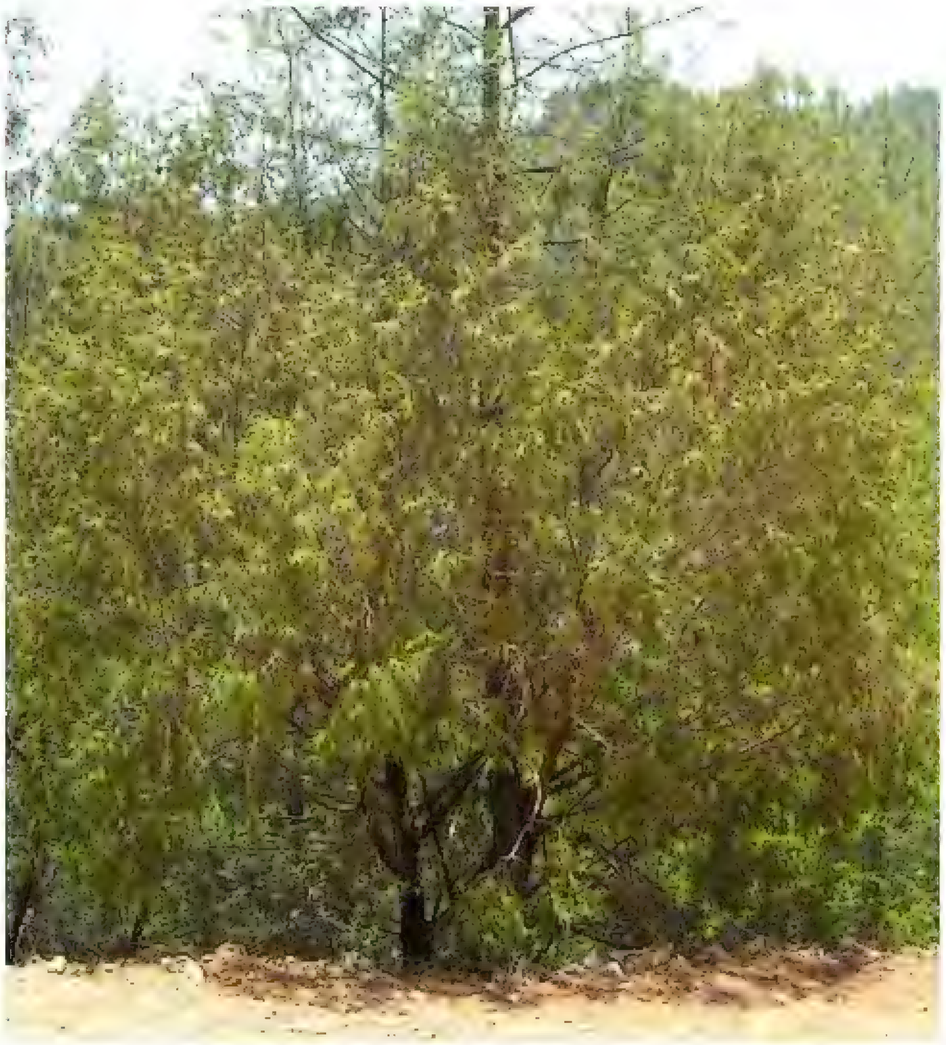

Fig. 8. *J. poblana* var. *decurrens*, habit.

The foliage is also more robust and planate (Fig. 9), than in *J. poblana*, Oaxaca (Fig. 10) and *J. p.* var. *decurrens* (Fig. 11). Notice the lack of blue bloom (glaucous) on the cones of the Nayarit juniper (Fig. 9).

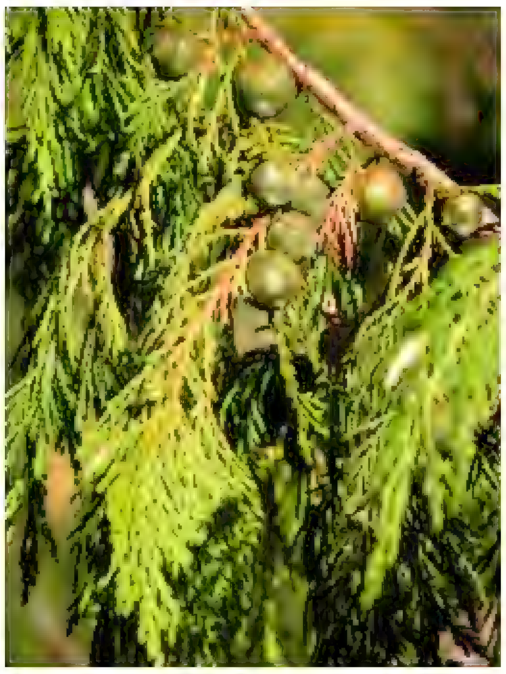

Fig. 9. Foliage of the Nayarit juniper.

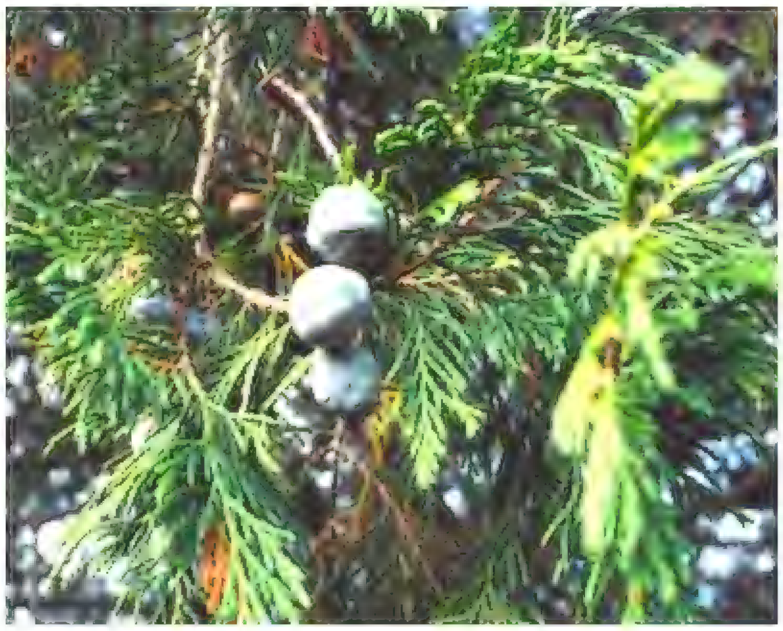

Fig. 10. Foliage and cones, *J. poblana*, S of Oaxaca.

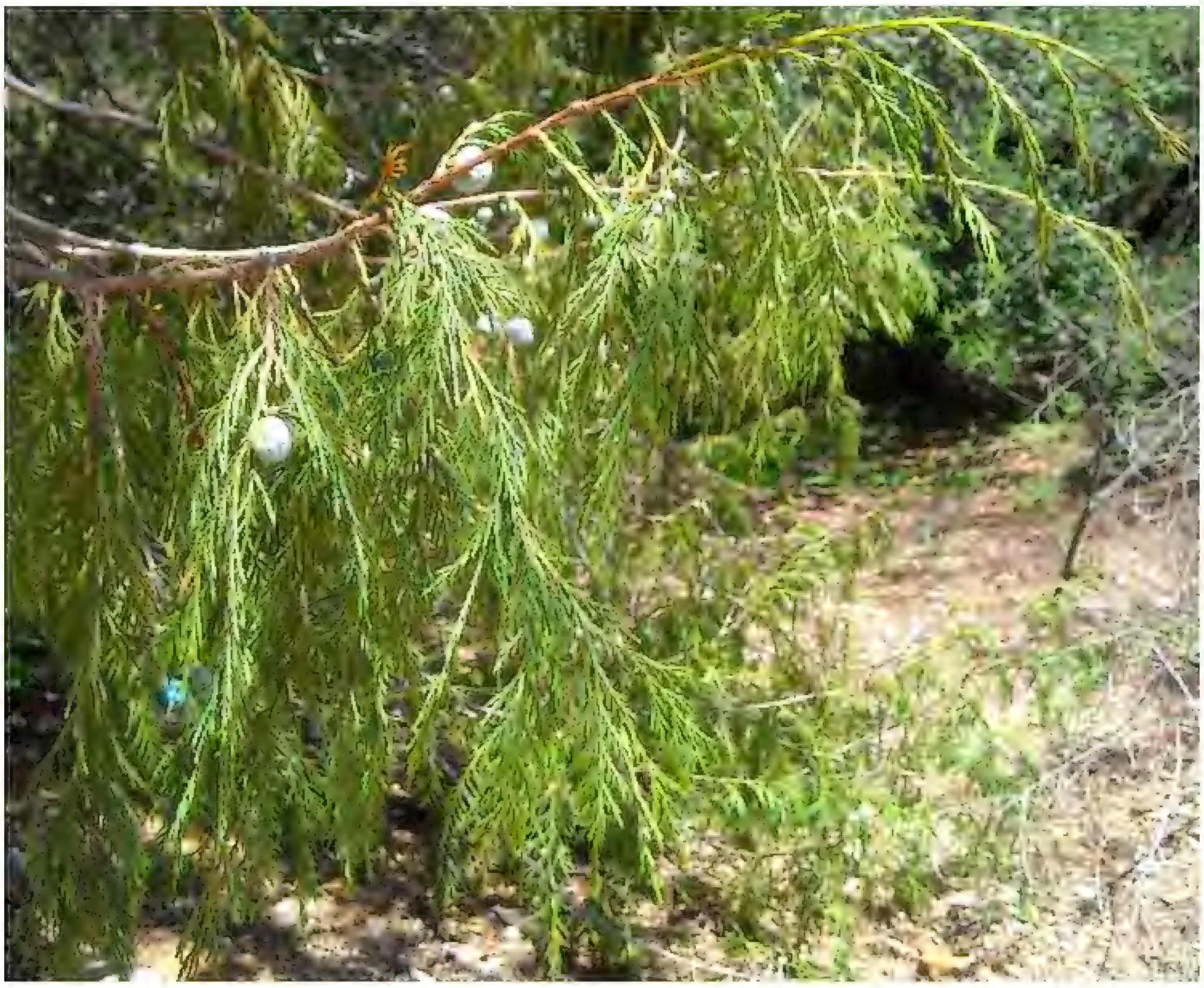

Fig. 11. Foliage and cones, *J. poblana* var. *decurrens*.

In summary, the Nayarit juniper differs in its leaf volatile oils, habit, aspects of its morphology, ecology and habitat (with elements of mesophytic and tropical deciduous forest), and is geographically disjunct, which support it recognition as a different lineage. However, molecular data show it closely clustering with *J. poblana*. Therefore, additional evidence is needed in order to resolve its taxonomic status.

ACKNOWLEDGEMENTS

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Lycianthes glabripetala (Solanaceae) a New Species of series *Strigulosae* from Queretaro, Mexico

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ABSTRACT

A new species, *Lycianthes glabripetala* E. Dean (Solanaceae), is described from the state of Queretaro, Mexico. *Lycianthes glabripetala* is closely related to *Lycianthes amatitlanensis* in series *Strigulosae.* It differs from that species in having curled trichomes on the underside of the leaves, a larger corolla, and nearly glabrous corolla lobes. Published on-line www.phytologia.org *Phytologia 100(1): 27-36 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: *Lycianthes,* Solanaceae, Mexico, Queretaro, taxonomy

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With approximately 150 to 200 species, the genus *Lycianthes* (Dunal) Hassler (Solanaceae) contains taxa in both the New and Old Worlds (Hunziker, 2001). The majority are distributed in the New World (from Mexico to Argentina), with about 43 taxa native to Mexico (ca. 18 endemic, one of them described here) (Villaseñor, 2016). The genus is the closest relative of the chili pepper genus *Capsicum* L. (Bohs & Olmstead, 1997; Särkinen et al., 2013; Walsh & Hoot, 2001); the German botanist Georg Bitter, who monographed the genus *Lycianthes*, first pointed out the similarity in calyx structure between *Lycianthes* and *Capsicum* (Bitter, 1919). In both genera, the five sepal lobes are truncated into a sleeve, below which may protrude five to ten appendages (commonly called calyx teeth). However, while *Capsicum* species have anthers that dehisce by longitudinal slits, the species of *Lycianthes* typically have poricidal anther dehiscence.

In his monograph on *Lycianthes*, Bitter created a series *Strigulosae*, for a cohesive group of 24 mostly South American species distinguished by their simple strigose hairs, shrub life form, and stellate corollas (Bitter 1919). In Mexico and Central America, the most commonly collected member of series *Strigulosae* is *Lycianthes amatitlanensis* (J.M.Coult. & Donn.Sm.) Bitter, originally described from Guatemala (Donnell Smith, 1904) but thought to occur from Mexico to South America (Benítez.de Rojas & D'Arcy, 1997; Villaseñor, 2016). While working on the treatment of *Lycianthes* for *"Flora del Bajio y de Regiones Adyacentes"* (Instituto de Ecología, A.C., ongoing), we were sent three collections of a new *Lycianthes* belonging to series *Strigulosae* from the state of Queretaro that differs from *L. amatitlanensis* in several characters (Table 1). It is described as a new species below.

METHODS

The species concept used in this taxonomic treatment is a morphological one (Cronquist, 1978), and the circumscription of the species described here is based on examination of herbarium specimens. For the species description, we measured three specimens sent to us as gifts from the Insituto de Ecología del Bajío (IEB) now housed at the UC Davis Center for Plant Diversity (DAV). Our observations were compared with measurements on specimens of other species of series *Strigulosae*, including type specimens, from: MO, NY, and US. Throughout this work, herbarium specimens with an accession number are cited with the herbarium acronym followed by the number. In cases where the specimen has an accession number and a barcode number, only the barcode number is cited. Herbarium codes were obtained from Index Herbariorum online (Theirs, Continuously Updated). In order to create maps, specimens were georeferenced using google maps online or Geolocate, an online software-mapping package (Rios and Bart 2010). Terminology here follows that of previous papers on this genus (Dean et al., 2017).

Lycianthes glabripetala E. Dean, sp. nov. TYPE: Mexico: State of Queretaro, Mpio. Landa, 10 Km al noreste de Agua Zarca, sobre camino a Neblinas, 1100 m, 23 Jun 1988, *Jerzy Rzedowski 46837* (holotype: DAV-217731 [Figure 1]; isotype: IEB-193504).

Diagnosis. This species is similar to *Lycianthes amatitlanensis* but differs in having diagnostic characters such as wavy to curved trichomes along the midvein of the abaxial side of the leaf and larger flowers that are nearly glabrous on the abaxial side of the corolla lobes.

Herbs to small shrubs, 0.5–2 m tall. **Indument** of off-white to tan, multicellular, simple, eglandular, acute, curved to wavy, usually appressed-antrorse [rarely patent] trichomes 0.25–1.25 mm long. **Stems** green when young, somewhat compressed upon drying, becoming light brown and woody with age, moderately to densely pubescent. Upper sympodial branching points mostly monochasial with a few dichasial branching points, the upper sympodial units 1–4 cm long, 1–2.5 mm in diameter, difoliate, the leaves usually geminate. **Leaves** simple, those of a geminate pair unequal in size, the larger ones with blades (4.5–) 8.5–13 × (1.8–) 2.5–4.5 cm, ovate to elliptic, the smaller ones with blades 1.3–4.5 × 0.8–2.1 cm, usually ovate, moderately to densely pubescent with soft, appressed to patent, often curved or bent trichomes to 1.25 mm long, these more numerous on the veins and appearing woolly along the midvein of the abaxial side, the major veins on large leaves (6–) 8–11 on each side of the midvein, the base cuneate, usually oblique (sometimes rounded in smaller leaves), the margin entire to delicately wavy, the apex acute to acuminate, the petioles absent or 0.1–1.5 cm long. **Flowers** 5-merous, solitary, axillary, the axes moderately to densely pubescent with usually appressed [rarely ascending] trichomes 0.25–1 mm long; peduncles absent; pedicels 9–15 mm long and arching in flower, 12–20 mm long and arching in fruit; calyx 2–2.5 mm long, 2.5–3 mm in diameter, obconic to narrowly campanulate, moderately pubescent with hairs similar to stem, the margin truncate to undulate, with 5–10 spreading linear appendages 0.5–2 mm long emerging 0.25–0.5 mm below rim; fruiting calyx slightly accrescent, widely bowl-shaped to plate-shaped, ca. 1.5–2 mm long, 4–6 mm in diameter, the teeth withering with age; corolla oriented horizontally to nodding, 1–1.2 cm long, campanulate, to reflexed, stellate, white, adaxial markings unknown, abaxially nearly glabrous (sparsely pubescent along the midvein); stamens equal, the filaments ca. 0.75–1 mm long, glabrous, the anthers ca. 3 mm long, lanceolate, abruptly narrowed at the tip, not connivent, the anther color unknown, the pores ovate, apical, opening upwards; pistil with glabrous ovary, the style ca. 8 mm long, linear, glabrous, widened distally into the stigma, the stigma capitate. **Fruit** a berry, ca. 3.2–6 mm long, 3.1–7 mm diameter, globose, orange at maturity, glabrous, lacking sclerotic granules; seeds ca. 30–60 per fruit, 1–1.2 × 0.5–1 mm, compressed but not flat, irregular in shape, semi-circular, depressed-ovate, triangular, or rhombic, orange, sometimes with one shallow ridge, the surface with shallow serpentine pattern.

Distribution. All three currently known *Lycianthes glabripetala* collections are from the Municipio of Landa on the east side of the state of Queretaro, Mexico (Figure 2).

Habitat. Shady canyons, slopes. Vegetation types include: bosque mesofilo de montaña (cloud forest), including oak forest at 1,040–1,100 m in elevation.

Phenology. Flowers present in June, and fruits present in July.

Etymology. The specific epithet describes the nearly glabrous corolla lobes, which differ from the closely related *Lycianthes amatitlanensis*.

Additional Specimens Examined. Mexico: State of Queretaro, Mpio. Landa, 1 Km al oriente del Puerto del Sabino, 1040 m, 4 Oct 1988, *H. Rubio 190* (DAV-214479, IEB-193287); Mpio. Landa, 1 Km al sureste de El Naranjo, 1050 m, 24 Jul 1989, *H. Rubio 909* (DAV-217888, IEB-193286).

DISCUSSION

The species of series *Strigulosae* are poorly studied, and we hesitated to describe this new species, given the work that still needs to be done on the series. However, as part of a project to prepare species descriptions for the Mexican and Central American species of *Lycianthes*, we have attempted to understand the morphological variation of the populations of series *Strigulosae* in this region and assign names to populations. We found that the populations of *Lycianthes glabripetala* are disjunct from known populations of *Lycianthes amatitlanensis* in Mexico (Figures 2 and 3). In addition, *Lycianthes glabripetala* differs from other members of series *Strigulosae* known from Mexico and Guatemala (Table 1) in combining woolly curved trichomes on the abaxial side of the leaves, a relatively large corolla (to 1.2 cm long), nearly glabrous surfaces on the abaxial side of the corolla lobes, and a pedicel length of 9–15 mm in flower and 12–20 mm in fruit (Figures 4a, 5a). The only other member of series *Strigulosae* known from Mexico, *L. amatitlanensis*, usually has straight trichomes that project at a 90-degree angle from the midvein of the abaxial leaf surface, corollas 0.5–0.8 cm long, and very evident long trichomes on the abaxial side of the corolla lobes with these hairs usually tufted at the tip of the lobe (Figures 4b, 5b). *Lycianthes inconspicua* Bitter, a member of series *Strigulosae* described from Guatemala (Bitter, 1919) with a distribution that likely ranges to Panama, can have flowers as long *L. glabripetala*, and has variable pubescence on the abaxial side of the corolla lobes, but it has longer pedicels (15–30 mm in flower and 30–35 mm in fruit), and it has delicate straight trichomes that are tightly appressed to the midvein of the abaxial leaf surface. *Lycianthes glabripetala* is known at this time from the highlands of central Mexico in the state of Queretaro in cloud forest vegetation above 1000 m in elevation; this habitat is similar to that of *L. inconspicua* but differs from the most common habitat of *L. amatitlanensis*, a species that is usually found below 1000 m in elevation, often below 500 m, in humid tropical forest.

ACKNOWLEDGEMENTS

We thank: Jean Shepard for her help with receiving loans at UC Davis; the IEB, MO, NY, and US herbaria for loans and access to specimens used in this project; DAV, MO and US for granting permission to scan the specimens used in Figures 1, 3, 4, and 5; Daniel McNair for photographing the leaf in Figure 5B for our poster on series *Strigulosae* and permitting us to crop and adapt his photograph for this publication; Victoria Sosa and Michael Nee for helpful comments which much improved the manuscript; Michael Nee for helpful suggestions on the morphology and evolution of series *Strigulosae*; NSF award 1457351 to Ellen Dean for working on species descriptions for *Lycianthes*; NSF award 1457351 for Research Experience for Undergraduates support for Mayra Huerta; and Shirley Tucker for salary support for Daniel McNair.

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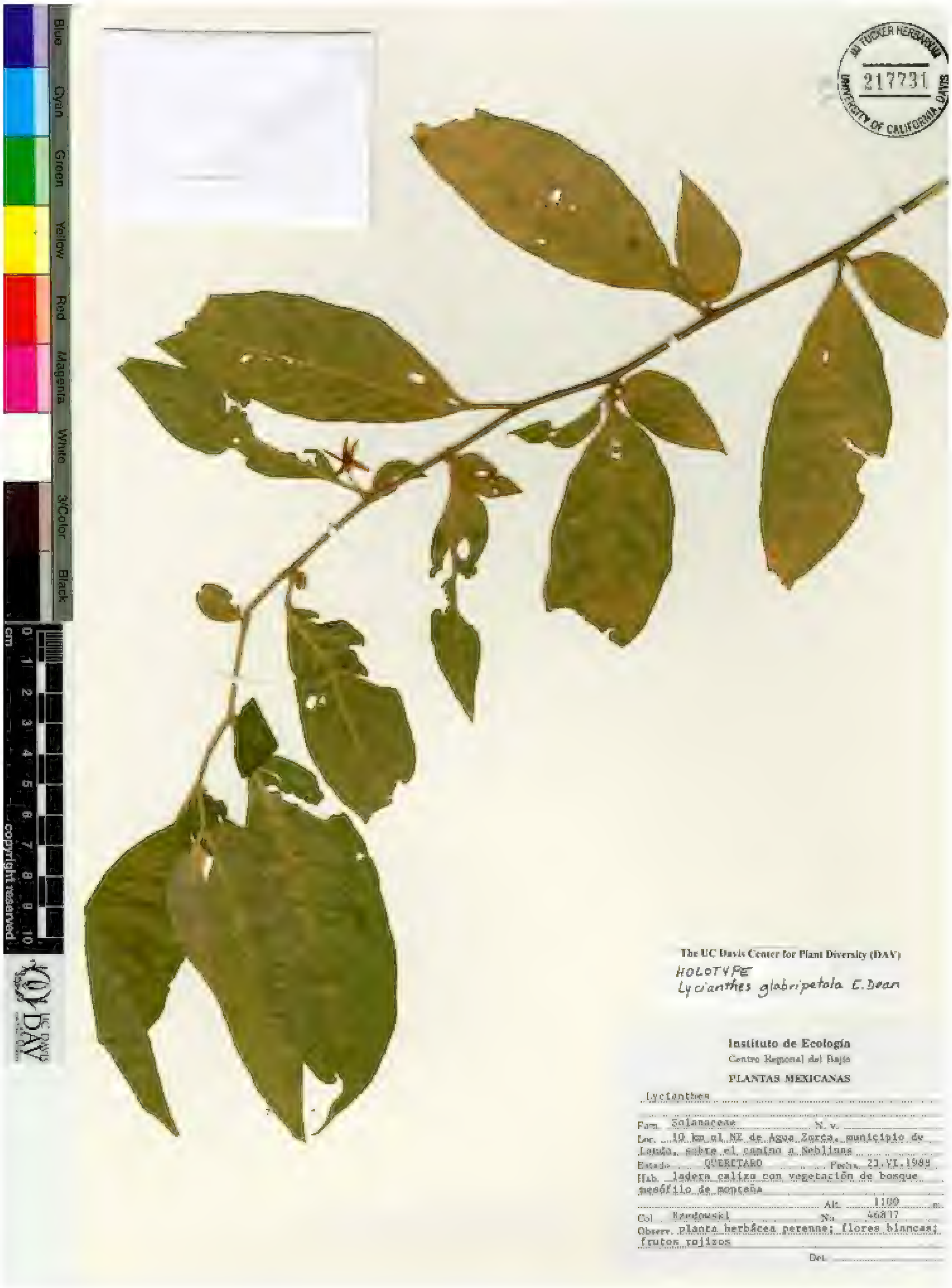
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HOLOTYPE
Lycianthes glabripetala E. Dean
Instituto de Ecología
Centro Regional del Bajío
PLANTAS MEXICANAS
Lycianthes
Fam. Solanaceae N. v.
Loc. 10 km al NE de Agua Zarca, municipio de Landa, sobre el camino a Neblinas
Estado QUERETARO Fecha 23.VI.1989
Hab. ladera caliza con vegetación de bosque mesófilo de montaña
Alt. 1100 m.
Col. Rzedowski No. 46837
Observ. planta herbácea perenne; flores blancas; frutos rojizos
Det.

Figure 1. Image of holotype of *Lycianthes glabripetala*, (*Rzedowski 46837*, DAV-217731). Use of specimen image courtesy of the UC Davis Center for Plant Diversity.

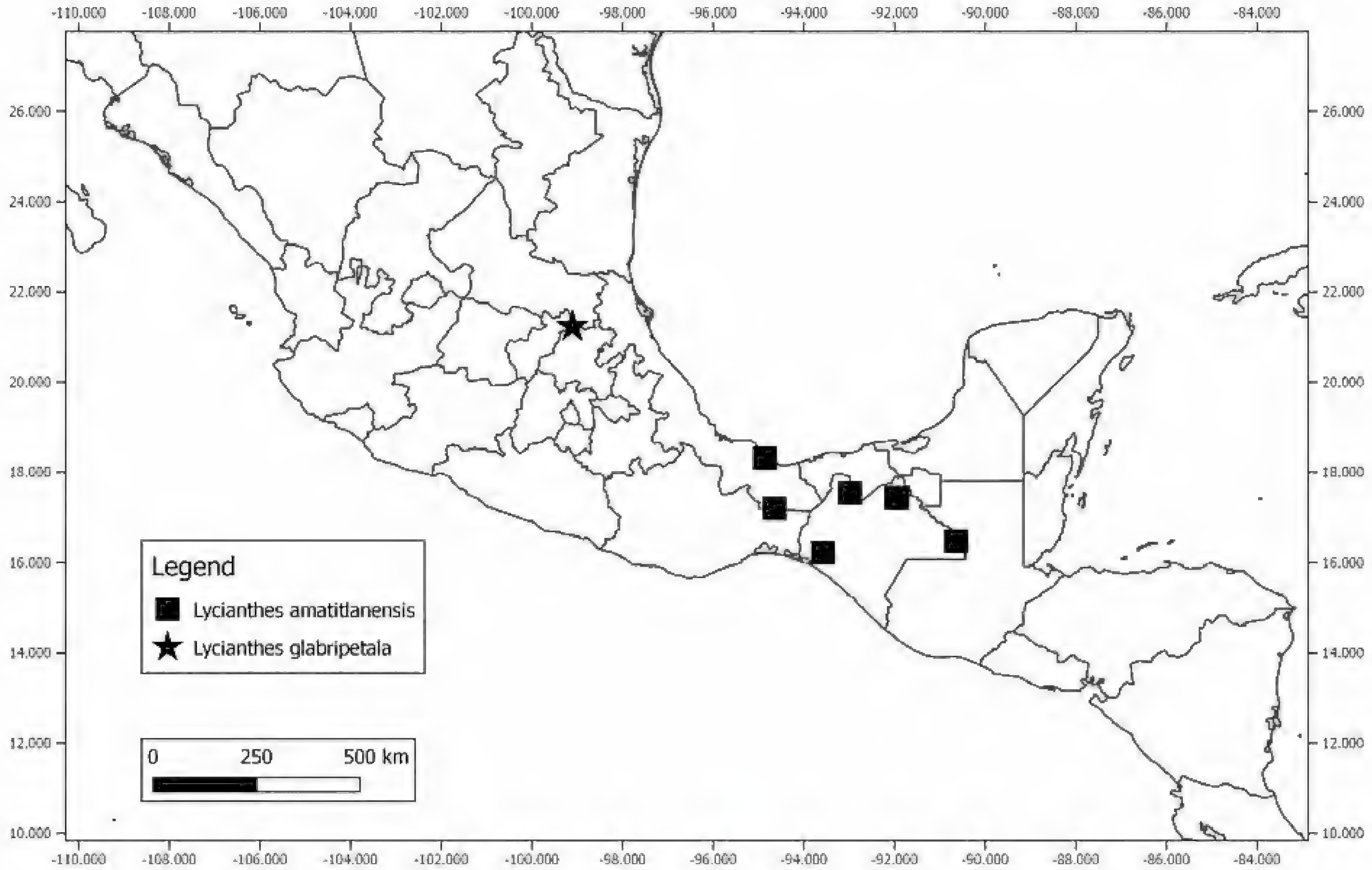
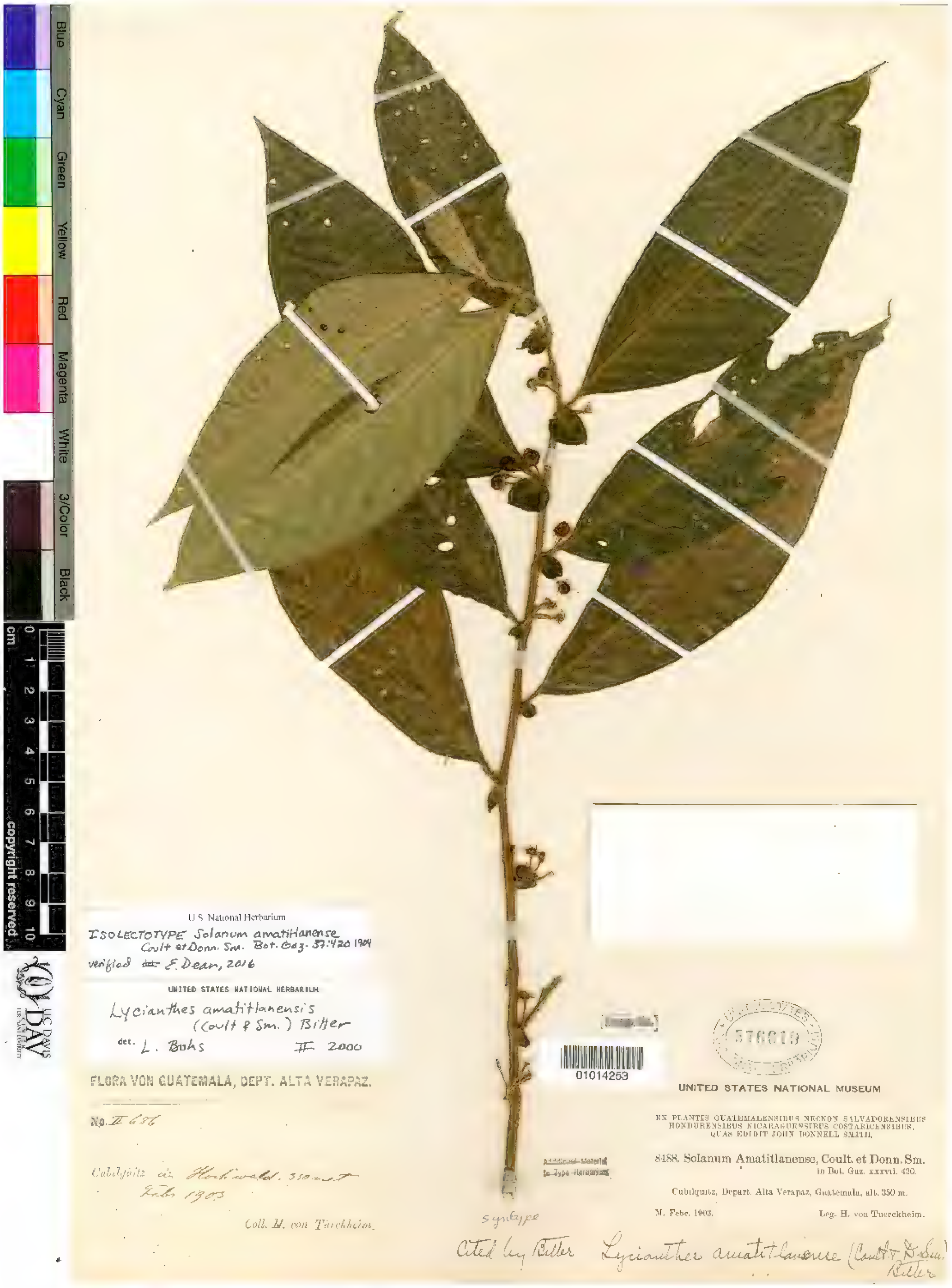


Figure 2. Map of known specimens of *Lycianthes glabripetala* and specimens of *Lycianthes amatitlanensis* in Mexico that have been confirmed by the first author.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ISOLECTOTYPE Solanum amatitlanense Coult et Donn. Sm. Bot. Gaz. 37:420 1904
verified ~~det~~ E. Dean, 2016
UNITED STATES NATIONAL HERBARIUM
Lycianthes amatitlanensis (Coult & Sm.) Bitter
det. L. Bohs II 2000
FLORA VON GUATEMALA, DEPT. ALTA VERAPAZ.
No. II 686
Cubilgüitz in Hochwald. 350 met
Febr 1903
Coll. H. von Türckheim.
01014253
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UNITED STATES NATIONAL MUSEUM
EX PLANTIS GUATEMALENSIBUS NECNON SALVADORENSIBUS HONDURENSIBUS NICARAGUENSIBUS COSTARICENSIBUS, QUAS EDIDIT JOHN DONNELL SMITH.
~~Additional Material to Type Herbarium~~
8488. Solanum Amatitlanense, Coult. et Donn. Sm.
in Bot. Gaz. xxxvii. 420.
Cubilquitz, Depart. Alta Verapaz, Guatemala, alt. 350 m.
M. Febr. 1903.
Leg. H. von Tuerckheim.
syntype
Cited by Bitter
Lycianthes amatitlanense (Coult & D. Sm.) Bitter

Figure 3. Image of isolectotype of *Solanum amatitlanense* Coult. & Donn.Sm. (*Lycianthes amatitlanensis*), (*von Tuerckheim 8488*, US-01014253). Use of specimen image courtesy of the Smithsonian Institution.

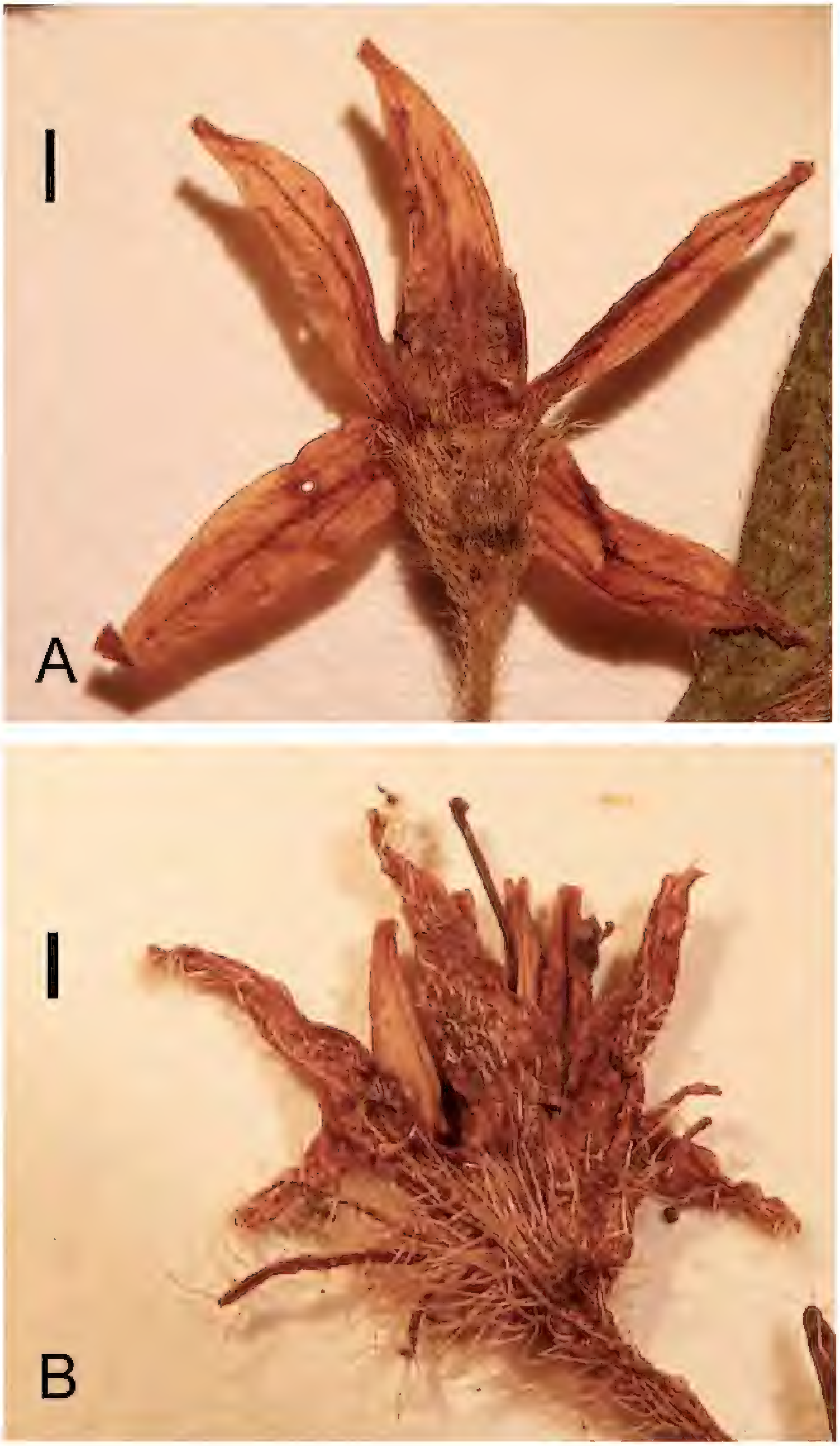

Figure 4. A. Scale bar equals 2 mm. Flower of *Lycianthes glabripetala* (*Rzedowski 46837*, DAV-217731) showing the relatively large corolla with nearly glabrous abaxial surfaces. B. Scale bar equals 1 mm. Flower of *Lycianthes amatitlanensis* (*D'Arcy 18034*, MO-4401624) showing the relatively small corolla with pubescent abaxial surfaces. Use of specimen images courtesy of the UC Davis Center for Plant Diversity and the Missouri Botanical Garden.

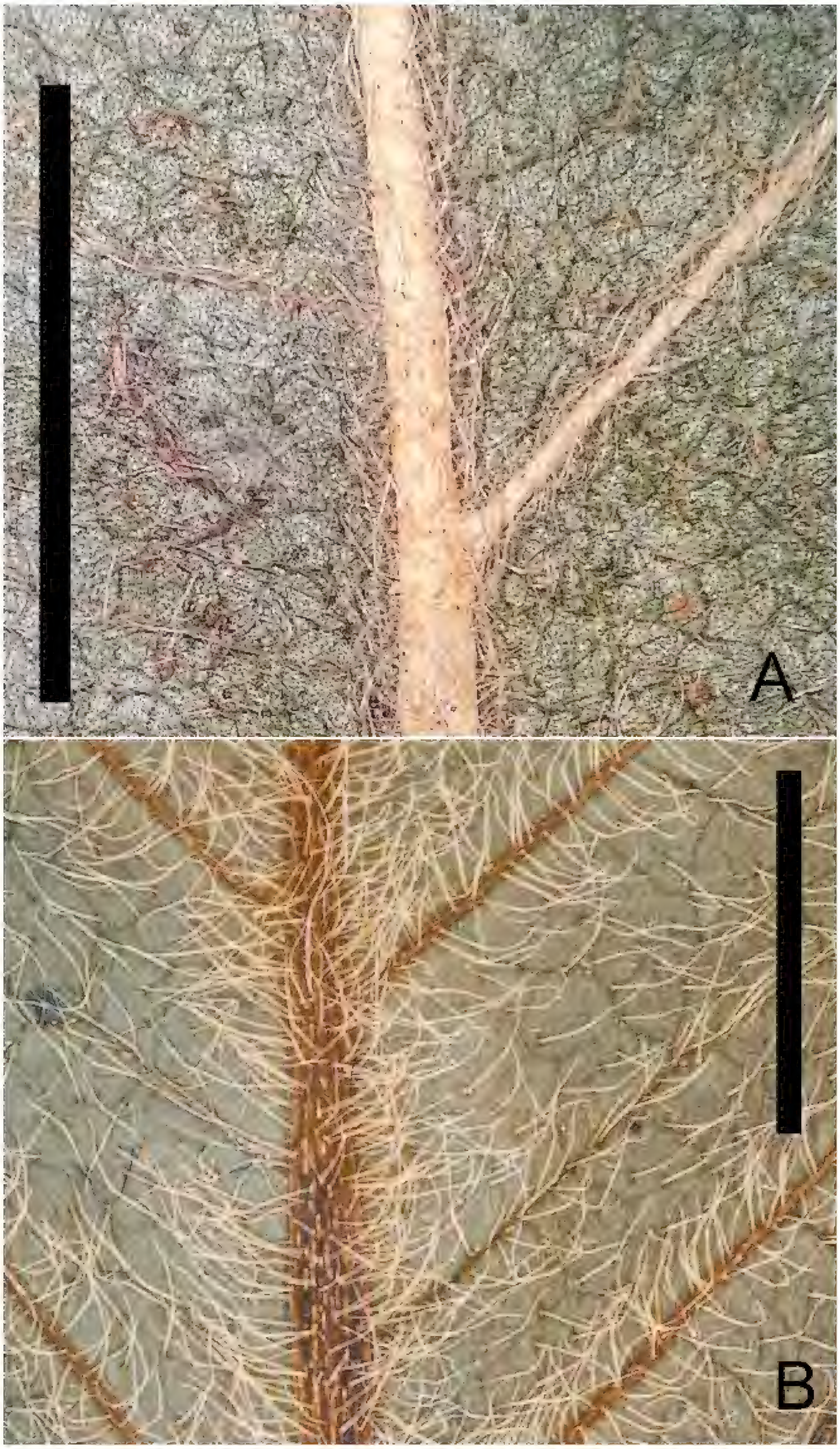

Figure 5. Scale bar equals 5 mm. A. Abaxial leaf surface of *Lycianthes glabripetala* (*Rubio 190*, DAV-217888) showing wavy to bent trichomes along midvein. B. Abaxial leaf surface of *Lycianthes amatitlanensis* (*von Tuerckheim 8488*, US-01014253) showing straight trichomes that project at a 90-degree angle from the midvein. Use of specimen images courtesy of the UC Davis Center for Plant Diversity and the Smithsonian Institution.

Table 1. Comparison of seven characters in *Lycianthes glabripetala*, *L. amatitlanensis*, and *L. inconspicua*.

Species	**L. glabripetala**	**L. amatitlanensis**	**L. inconspicua**
Trichomes on abaxial side of the leaf	Bent to wavy, appearing woolly along the midvein	Mostly straight, projecting at a 90-degree angle from the midvein	Mostly straight, appressed to the midvein
Number of major veins on large geminate leaf	(6–) 8–11	(7–) 10–22	6–12
Pedicel length	9–15 mm in flower; 12–20 mm in fruit	4–12 mm in flower; 6–16 mm in fruit	15–30 mm in flower; 30–35 mm in fruit
Calyx appendage number	5–10	10	10
Corolla length	1–1.2 cm	0.5–0.8 cm	0.8–1.2
Pubescence on abaxial side of corolla lobes	Sparse, short trichomes; nearly glabrous, not tufted at tip	Moderately to densely pubescent with long trichomes, these tufted at lobe tip	Sparsely to densely pubescent with wavy trichomes, not tufted at lobe tip
Seed length	1–1.2 mm	0.9–1.1 mm	1–1.5 mm

Survey of cotton (*Gossypium* sp.) for non-polar, extractable hydrocarbons for use as petrochemicals and liquid fuels

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ABSTRACT

A survey of USDA cotton germplasm accessions, grown with supplemental underground drip irrigation to achieve best yields at College Station, TX, found % HC yields from 7.35 % to 3.14 %. Leaf dry weights (DW) varied about 2-fold from very large leaves: TX-1196 (1.59 g), TX-1757 (1.43), TX-1192 (1.26) to small leaves: SA-1427 (0.59 g), STD-10 (0.64), SA-1232 (0.67). Yields as g HC/ g DW leaf ranged from 0.080 g to 0.028 g. None of the accessions in this survey (2017) were in the 70th percentile of the highest thirty 2016 accessions (7.37 - 13.73 %). It appears that the high HC yielding quantities found in the 2016 survey were atypical and may be due to some unknown factor such as insect and/ or disease damage that caused an induction of defensive chemicals. The 2017 survey seems to be more typical of HC yields in cotton. Additional research is needed to determine the factor(s) that caused the unusually high HC yields in the 2016 test plots. Published on-line www.phytologia.org *Phytologia 100(1): 37-44 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* sp., yields of hexane extractable leaf hydrocarbons, petrochemicals, liquid fuels.

The domestication of cotton has a complex history (see Wendel, J. F. and C. E. Grover, 2015). Recently, we (Adams et al. (2017a) reported on hydrocarbon (HC) yields of 30 cotton accessions representing photoperiodic and non-photoperiodic forms of two species grown with supplemental underground drip irrigation to achieve best yields at College Station, TX. They reported very high % HC yields in four accessions yielding 11.34, 12.32, 13.23 and 13.73% (Table 1). Per plant HC yields varied from 0.023 to 0.172 g/ g leaf DW. Hopi had a high % HC yield (10.03%), but it was the lowest per plant yield (0.023 g/ g leaf DW). In contrast, China 86-1 had the second highest % HC yield (13.23%) and also had the highest per plant yield (0.172 g). The correlation between % HC yield and avg. leaf DW was non-significant (0.092). They concluded that it appears that one might breed for both % HC yield and leaf DW in cotton.

Principal Coordinate Analysis (PCoA), utilizing 597 SSR bands, of the 30 accessions revealed the accessions are divided into *G. barbadense* and *G. hirsutum* (Fig. 1, left and right) (see Hinze et al., 2016 for further details on molecular marker analysis). The *G. barbadense* samples (8) are all improved accessions. The samples of *G. hirsutum* contain both wild and improved accessions forming a very loose group, but the wild accessions are mostly found in the upper-right quadrant of the ordination (Fig. 1).

Utilizing the g HC/ g leaf DW data, the high HC yielding accessions are clearly clustered in a tightly grouped set of improved accessions (Fig. 1, dashed oval). Plotting the high and highest yielding samples revealed that all three of the high yielding samples (SA-1181, SA-1403, SA-2269, top 13%) and the highest yielding individual (SA-1419, top 3%) are found in that group (Fig. 1, dashed oval). The discovery of the highest yielding individuals in a group of improved accessions is surprising, in view of the selection for increased cotton seed and fiber yields.

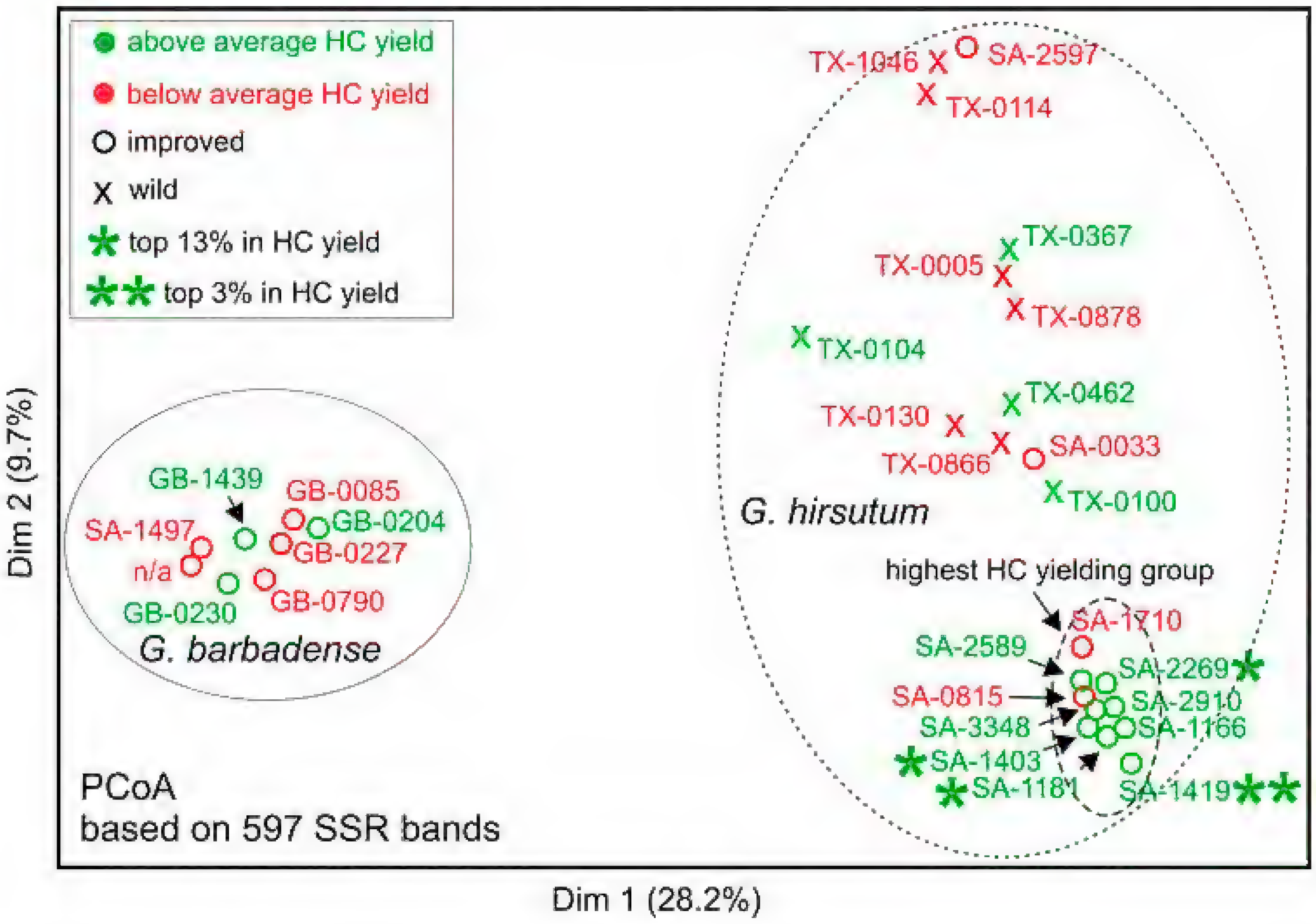

Figure 1. Principal Coordinate Analysis (PCoA) based on 597 SSR bands. The percent of variance accounted for among accessions is given on Dim 1 and Dim 2. See text for discussion.

It is also surprising that none of the wild accessions had high yields, although TX-0100 had a high % yield (10.72%), but having smaller leaves resulted in a moderate total g HC/ g leaf DW yield (Table 1). It is interesting that genetically (by SSR data), TX-0100 is ordinated nearest of any other wild accessions to the high HC yielding group (Fig. 1). It may be that back-crossing TX-0100 with SA-1419 might produce some useful progeny in the future.

Because the high HC yielding accessions were clustered in a small region of *G. hirsutum* (Fig. 1), it seemed promising to grow additional related accessions in the summer of 2017 to determine if other high yielding accessions might be discovered. This paper reports on the HC yields from 26 additional accessions grown in the same plot area as the previous 30 accessions grown in 2016.

MATERIALS AND METHODS

Plant Materials:

Cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, 30 37' 5.00" N, 96 21' 50" W, 354 ft., subsurface drip irrigation, sandy soil, annual rainfall 40". Fifteen total leaves, sampled as 3-4 mature leaves (from the 4th and 7th nodes below the growing point) from each of 4-5 cotton plants, were bulked for an accession sample. The accessions were primarily non-photoperiodic landraces (one landrace was photoperiodic) and obsolete cultivars representing the most commonly grown commercial tetraploid cotton species, *G. hirsutum*. Cotton plants have an indeterminate growth habit and, therefore, vegetative and reproductive development occur at the same time. At the time of sampling, each plant had matured to the formation of green bolls while at the same time the plant had squares and flowers. These accessions were collected worldwide and are maintained by the USDA National Cotton Germplasm Collection in College Station, TX.

Leaves were ground in a coffee mill (1mm). Three grams of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. The soxhlet correction factor (sCF) was determined to be 1.14. All shaker extraction yields were corrected to oven dry weight (ODW) by multiplication of 1.085. Thus, the total CF was 1.24 (1.14 x 1.08).

RESULTS

The survey of USDA germplasm cotton accessions grown with supplemental irrigation at College Station, TX, found (Table 2) that % HC yields ranged from 7.35 % (STD-08) to 3.14 % (SA-2356).

Leaf dry weights (DW) varied about 2-fold (Table 2) from very large leaves: TX-1196 (1.59 g), TX-1757 (1.43), TX-1192 (1.26) to small leaves: SA-1427 (0.59 g), STD-10 (0.64), SA-1232 (0.67).

Yields as g HC/ g DW leaf ranged from 0.080 g (TX-1192), 0.079 (STD-08), 0.076 (TX-1196) to about 1/3 as much: SA-1427 (0.029 g) and SA-2356 (0.028 g).

None of the accessions in this survey (2017) were in the 70th percentile of the thirty 2016 accessions (7.37 - 13.73 % HC, Table 1). However, 2016 appears to be an unusual year for HC production at College Station. Four accessions were grown at College Station, TX in both 2016 and 2017 (Table 3). Notice (Table 3) that in 2016, leaf DW was larger for SA-1403 and SA-1419, but smaller for SA-1181 and SA-2269. The % HC yields were all higher in 2016 and often, much higher, cf. S-1181, 12.31 % (2016) vs. 6.41 % (2017). Due to the much higher % HC yields and small differences in leaf weights, the mg HC/ leaf wt was also much higher in 2016. We have yet to find a factor to explain these differences between the years. It seems likely that 2016 was a time of stress induction of HC by damage from insects, disease or some other vector more recently, greater focus has been on inducible plant defenses (Chen 2008; Opitz, Kunert and Gershenzon, 2008; Pare and Tumlinson, 1997, 1998; Turlings, et al. 1995).

It seems relevant to examine the work of Stipanovic, Bell and Benedict (1999) who reviewed the defensive role of pigment gland constituents in cotton. They found that cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed that these gland constituents can be rapidly synthesized in response to pathogens.

Chen (2008) also found that some constitutive chemicals may be increased to even higher levels after insect attack. Opitz, Kunert and Gershenzon (2008) analyzed the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They showed that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments. In addition, they reported that herbivory or mechanical damage in older leaves led to terpenoid increases in younger leaves. It might be noted that normally plants lose their lower (older) leaves and these leaves are usually damaged by insects and diseases. Opitz et al. (2008) found the composition of the terpenoid mixture did not significantly differ in response to herbivore, mechanical damage or jasmonic acid treatments.

Table 3. Comparison g DW/ leaf, % HC yield, and mg HC/ g DW leaf for SA-1181, SA-1403, SA-1419 and SA-2269 grown in a common test plot at USDA, College Station in successive years (2016, 2017).

	g DW/ leaf		% HC yield		g HC/ g DW leaf	
accession	2016	2017	2016	2017	2016	2017
SA-1181	0.96	1.21	12.32	6.41	0.119	0.078
SA-1403	1.46	1.11	9.08	7.45	0.133	0.080
SA-1419	1.30	0.99	13.23	6.41	0.172	0.063
SA-2269	1.24	1.36	11.09	5.94	0.138	0.082

Turlings et al. (1995) showed that plants injured by herbivores emit chemical signals that attract and guide the herbivores' natural enemies to the damaged plants. Thus, indirectly, injured plants send out a "SOS" signal for help against herbivores. Pare and Tumlinson (1997) also confirmed this phenomenon in a series of experiments on cotton using beet army worms and mechanical damage to leaves.

To examine the environmental component of % HC yields, Adams, et al. (2017b) compared % HC yields of SA-1181, SA-1403, SA-1419 and SA-2269 grown in a greenhouse to the yields from 2016 samples from College Station, TX. Adams et al. (2017b) concluded that the lower % yields (Fig. 2) in protected conditions (i.e., greenhouse) seems to imply the genotype is particularly affected by insects, diseases, water stress, etc. that apparently induced increased HC production in the field at College Station in 2016. We have added the % HC yields from the 2017 field plot samples grown at College Station (Fig. 2, crosshatched bars). For SA-1181, SA-1419, and SA-2269, the 2017 field yields are more like the greenhouse yields than the 2016 field yields (Fig. 2). However, SA-1403 has the field yields more similar (9.08, 7.45) than the greenhouse yield (Fig. 2). These data suggest that 2016 was an atypical year for the production of HC in the College Station plot.

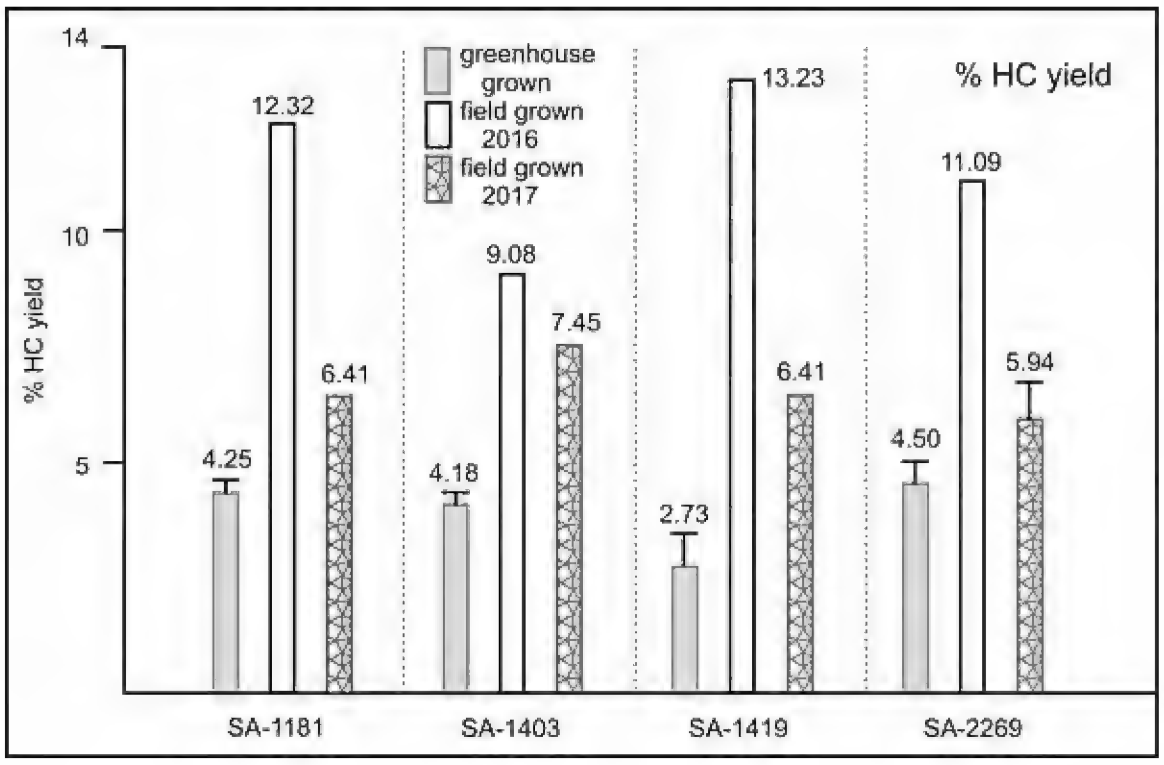

Fig. 2. Comparison of % yield of HC from greenhouse vs. field grown plants (2016 and 2017, College Station, TX). Modified from Adams et al. (2017b).

We considered that plant maturity might have been different between the samplings in 2016 and 2017, so a second harvest was conducted in 2017 for accession SA-2269. There was a very highly significant lowering of g DW leaf weight in the second sampling (10/2/2017, Table 4). The % HC yields were non-significant between sampling dates (Table 4). The mg HC/ g DW leaf was very highly significantly lower in the later (10/2/2017) harvest (Table 4, 0.082 mg, 0.047 mg) and this is near the magnitude seen in the 2016-2017 data. However, neither of these sampling dates (Table 4) explains the very large HC yields in the 2016 samples.

Table 4. Effects of plant maturity on the biomass (g DW/ leaf), % HC yield, and mg HC/ g DW leaf for SA-2269 harvested on 6/28/2107 (no bolls opened) vs. 10/2/2017 (most bolls opened). *** = very highly significant, ns = non significant.

accession date harvested	maturity stage	g DW/ leaf	% HC yield	g HC/ g DW leaf
SA-2269 6/28/2017	no bolls opened	1.36	5.97	0.082
SA-2269 10/02/2017	most bolls opened	0.89	5.39	0.047
	t-value	6.45	0.90	3.89
	probability	0.0001	0.387	0.0025
	significance	***	ns	***

It is instructive to graph leaf biomass, % HC yields, and g HC/ g leaf DW for SA-2269 onto a graph (Fig. 3) of these variables for commercially grown Fibermax 1320 (Adams et al. 2017b). Leaf biomass and g HC/ g leaf DW both decline from bolls maturing to bolls opened stages (Fig. 3) as also found in Fibermax 1320. However, the % HC yield for SA-2269 was not significantly different during that period, whereas, Fibermax 1320 had a barely significant increase (Fig. 3).

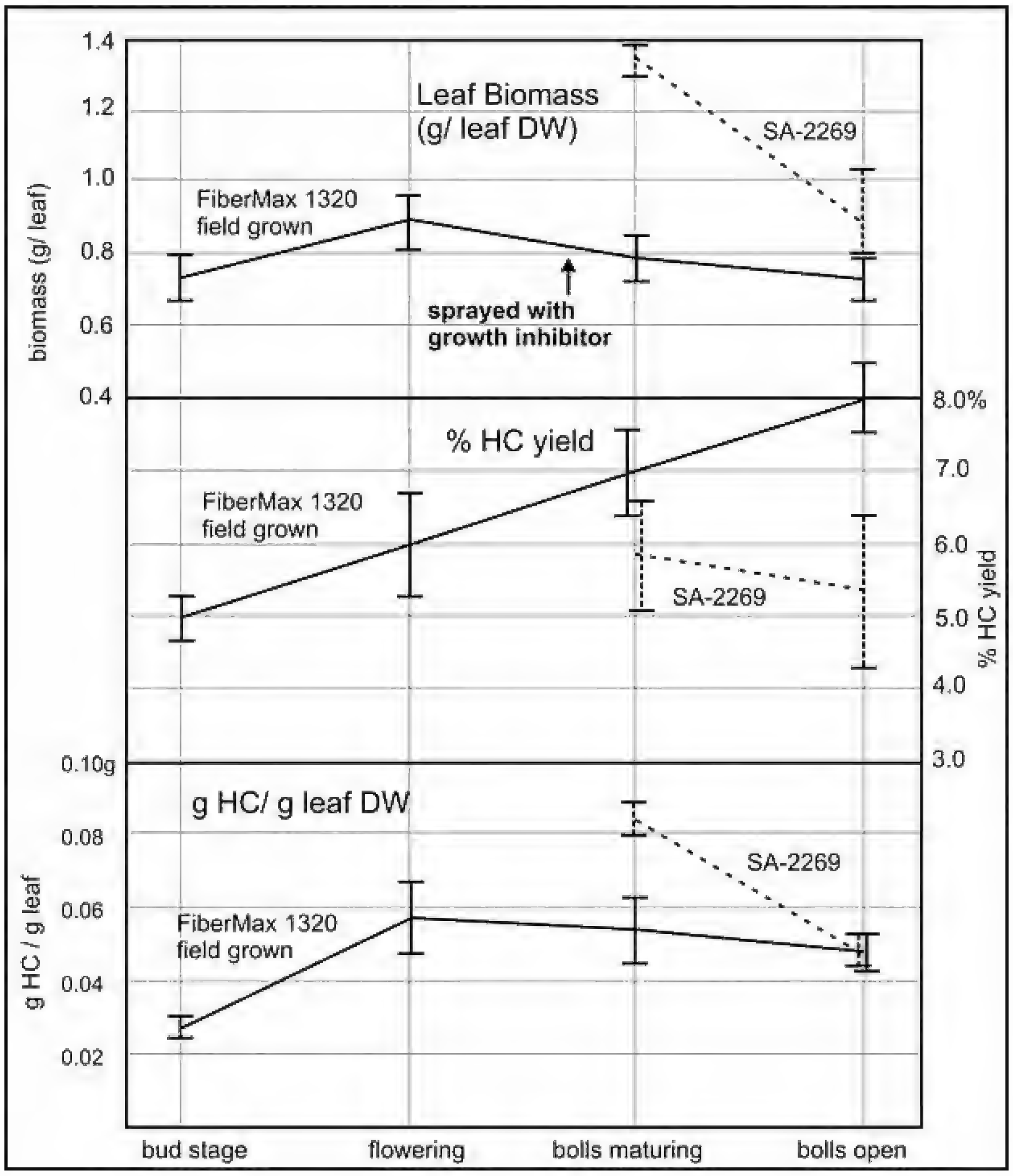

Figure 3. Ontogenetic variation in leaf biomass, % HC yields, and g HC/ g leaf DW in commercially grown Fibermax 1320 (from Adams et al. (2017b). These variables are also graphed for SA-2269 grown at USDA, College Station, TX (dashed lines). The bar lines are 2 standard errors of the mean.

There was considerable variation among the SA-2269 plants (Table 5) . Among the plants sampled with no bolls opened (the normal time to sample) the % HC yields ranged from 4.840 % to 7.44 %. This is similar to range of variation found in samples with most bolls opened (3.72 % to 7.65%, Table 5).

Table 5. Variation among plants for leaf weight, % yield HC, and g HC/ g leaf weight.

accession	source name	g DW 1 leaf	% yld	g/ g 1 leaf	# plants	coll date
SA-2269	SA-2269/TM1 (1-1)	1.44	6.53	0.094	5	06/28/2017
SA-2269	SA-2269/TM1 (1-2)	1.47	6.32	0.093	5	06/28/2017
SA-2269	SA-2269/TM1 (1-3)	1.47	7.44	0.109	5	06/28/2017
SA-2269	SA-2269/TM1 (1-4)	1.21	4.62	0.056	5	06/28/2017
SA-2269	SA-2269/TM1 (2-1)	1.23	4.84	0.060	5	06/28/2017
SA-2269	SA-2269/TM1 (2-2)	1.26	5.62	0.071	5	06/28/2017
SA-2269	SA-2269/TM1 (3-1)	1.45	6.41	0.093	5	07/06/2017
SA-2269	no bolls opened, Avgs =	1.36	5.97	0.082		
SA-2269	SA-2269/TM1 (1-1)	0.69	5.99	0.041	5	10/2/2017
SA-2269	SA-2269/TM1 (1-2)	0.80	4.71	0.038	5	10/2/2017
SA-2269	SA-2269/TM1 (1-3)	1.08	3.72	0.040	5	10/2/2017
SA-2269	SA-2269/TM1 (1-4)	0.94	5.24	0.049	5	10/2/2017
SA-2269	SA-2269/TM1 (2-1)	0.85	7.65	0.065	5	10/2/2017
SA-2269	SA-2269/TM1 (2-2)	1.00	5.00	0.050	5	10/2/2017
SA-2269	most bolls opened, Avgs =	0.89	5.39	0.047		

CONCLUSION

This survey found a few moderately high HC yielding cotton accessions, but not as promising as found in the 2016 survey. However, it should be noted that growth of four of our best accessions in successive years (2016, 2017) failed to generate consistent yields of HC. The atypically high HC yields in the College Station plot of 2016 are under further investigation.

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Table 1. Screening results of 30 cotton accessions, grown in 2016 in a test plot at USDA, Crop Germplasm Research, College Station, TX. From Adams et al. (2017).

Lab acc Source	USDA identifier	g avg leaf DW (# plants)	% yield HC	g HC yield/ g leaf DW
14994, U12, Christidis 53D7	SA-1166	0.706 (4)	13.73 ++Hi	0.097
14997, U15, China 86-1	SA-1419	1.300 (4)	13.23 ++	0.172 ++Hi
14995, U13, Acala SJ-1	SA-1181	0.962 (4)	12.32 ++	0.119 +
15002,U20,Vir-7080Col.Macias17	SA-3348	0.896 (4)	11.34 ++	0.102
14998, U16, TM 1	SA-2269	1.244 (4)	11.09 ++	0.138 +
15001, U19,TAM 91C-34	SA-2910	1.006 (4)	10.85 +	0.109
15004, U22,Latifolium, wild	TX-0100	0.894 (5)	10.72 +	0.096
14985, U3, Nevis 81	GB-0227	0.728 (4)	10.36 +	0.041
14999, U17, KL 85/335	SA-2589	0.812 (4)	10.25 +	0.083
14992, U10, Hopi	SA-0033	0.266 (4)	10.03 +	0.023 Low
15009, U27,Richmondi, wild	TX-0462	0.973 (5)	9.93	0.097
14988, U6, Tadla 2	GB-1439	1.106 (4)	9.70	0.107
15005, U23,Latifolium, wild	TX-0104	0.967 (5)	9.25	0.089
14996, U14, 3010	SA-1403	1.463 (4)	9.08	0.133 +
15000, U18, KLM-2026	SA-2597	0.802 (4)	9.02	0.072
15007, U25,Morrili, wild	TX-0130	0.830 (5)	8.67	0.072
15010, U28,Marie-galante, wild	TX-0866	0.511 (5)	8.05	0.041
14990, U8, Pima S-5	SA-1497	0.995 (4)	7.92	0.079
14993, U11, Mexican #68	SA-0815	0.994 (4)	7.92	0.079
15003, U21,Palmeri, wild	TX-0005	0.398 (5)	7.92	0.032
14984, U2, Mono 57	GB-0204	1.360 (4)	7.37	0.100
14986, U4, Ashmouni Giza 32	GB-0230	1.128 (4)	7.37	0.083
15008, U26,Marie-galante, wild	TX-0367	1.289 (5)	7.37	0.095
14989, U7, 3-79	na	0.720 (4)	7.06	0.051
14987, U5, Ashabad 1615	GB-0790	0.866 (4)	7.01	0.061
14991, U9, TAM 87N-5	SA-1710	0.764 (4)	6.64	0.051
15006, U24,Punctatum, wild	TX-0114	0.815 (5)	6.33	0.052
14983, U1, Tanguisw LMW 12-40	GB-0085	1.335 (4)	5.97	0.080
15011, U29,Marie-galante, wild	TX-0878	0.692 (5)	4.50	0.031
15012, U30,Yucantanense, wild	TX-1046	0.728 (5)	3.29 Low	0.024 Low

Table 2. Screening results of 26 cotton accessions, grown in 2017 in a test plot at USDA, Crop Germplasm Research, College Station, TX

accession	source name	g DW 1 leaf	% HC yield	g/ g 1 leaf	# plants	collection date
STD-08	AllTexAtlas	1.07	7.35 HI	0.079 Hi	4	06/28/2017
SA-2260	PD 93007	.94	7.28 HI	0.068	5	07/06/2017
TX-1205		.75	6.66	0.050	5	06/28/2017
SA-0965	PLAINS	1.10	6.66	0.074	5	07/06/2017
STD-10	CAMDE	.64 Low	6.66	0.042	5	06/28/2017
SA-1212	MEXICO 910	1.06	6.49	0.069	5	07/06/2017
SA-0369	D AND PL 10-1	1.07	6.41	0.069	5	07/06/2017
TX-1192		1.26 Hi	6.32	0.080 Hi	5	06/28/2017
SA-0300	ROWDEN #2	.93	6.29	0.059	5	07/06/2017
STD-06	Acala1517-99	.83	5.87	0.049	5	06/28/2017
SA-2330	MARICO (SMOOTH)	1.15	5.83	0.067	5	07/06/2017
SA-3777	PAYMASTER 892	1.04	5.74	0.060	5	07/06/2017
SA-1349	IAC 18	1.11	5.74	0.063	5	07/06/2017
SA-1232	AC 134 CB 4029	.67 Low	5.36	0.036	4	07/06/2017
STD-02	FM832	.88	5.33	0.047	5	06/28/2017
SA-0825	MEXICAN #102	.82	5.24	0.043	5	07/06/2017
STD-01	AcalaMaxxa	1.08	5.24	0.057	5	06/28/2017
SA-1427	LONG FIBER #2	.59 Low	4.90	0.029	5	07/06/2017
TX-1196		1.59 Hi	4.84	0.076	5	06/28/2017
SA-1184	COKER 310	1.20	4.84	0.058	5	07/06/2017
SA-2910	TAM 91C-34	.80	4.34	0.035	5	07/06/2017
SA-1230	73 CB 3992	.76	4.25	0.033	5	07/12/2017
SA-1465	DES 422	1.00	4.13	0.041	5	07/06/2017
STD-07	SG747	.92	3.59	0.033	5	06/28/2017
TX-1757		1.43 Hi	3.50	0.050	4	07/12/2017
SA-2356	FUNTUA FT-5	.90	3.14 Low	0.028 Low	5	07/06/2017

The composition of the leaf essential oils of *J. sabina* var. *balkanensis*: chemotypes high in trans-sabinyl acetate and methyl eugenol discovered in three natural populations

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Abstract

The composition of the leaf oil of *J. sabina* var. *balkanensis* is reported. *J. sabina* var. *balkanensis* oils contained chemotypes in all three populations examined. The Greece population contained chemotypes in the concentration of methyl eugenol (13.2%, trace) and elemicin (6.0%, trace). Both chemotypes were low in trans-sabinyl acetate. The eastern Rhodopes and Rila Mtns. had chemotypes for high and low trans-sabinyl acetate (39.9, 0.4%, and 39.8, 0.4%, respectively). Associated with these chemotypes were: low-high α-pinene (7.5, 41.2%, and 3.7, 41.3%); and high-low trans-thujone (12.7, 0.2% and 6.7, 0.1%), respectively. A comparison is presented of the compositions of the leaf essential oils of *J. sabina* var. *sabina*, from Pyrenees, Switzerland and Kazakhstan. The composition of the volatile leaf oils of *J. s.* var. *balkanensis* do not appear to be different in any major components from that of *J. s.* var. *sabina*, Switzerland. Due to the chemotypes, it is not possible to discern any geographic differentiation among the var. *balkanensis* populations sampled. Published on-line www.phytologia.org *Phytologia 100(1): 45-50 (Mar 16, 2018)*. ISSN 030319430.
KEY WORDS: *Juniperus sabina* var. *balkanensis*, volatile leaf oils, terpenes, composition. *J. sabina.*

Recently, Adams, Schwarzbach and Tashev (2016) discovered a new variety of *Juniperus sabina* L., *J. s.* var. *balkanensis* R. P. Adams & A. N. Tashev from three locations in Bulgaria and one population in northern Greece. This new variety appears to morphologically nearly identical to *J. s.* var. *sabina*. var. *balkanensis* appears to have the nrDNA of *J. sabina*, but has the chloroplast of *J. thurifera*. It appears var. *balkanensis* is of ancient hybrid origin (Adams, Schwarzbach and Tashev, 2016). Additional sampling revealed var. *balkanensis* also occurs in far western Turkey (Adams et al. 2017) and in the Croatia-Macedonia region (Adams et al. 2018, in press).

Previously, Adams, Nguyen and Liu (2006) reported on the composition of the leaf oils from seven populations of *J. sabina* and one population of *Juniperus sabina* var. *arenaria* (E. H. Wilson) Farjon (now *J. davurica* var. *arenaria* (E. H. Wilson) R. P. Adams) as well as the oils of *J. chinensis* L. and *J. davurica* Pall. They found considerable differentiation in populations of *J. sabina* from the Iberian peninsula and far eastern populations (Kazakhstan, China). The amounts of cedrol, citronellol, safrole, trans-sabinyl acetate, terpinen-4-ol and trans-thujone were found to be polymorphic in several populations.

The leaf oils of the aforementioned species, except var. *balkanensis*, have been reported, and reviewed: *J. chinensis* (Adams, Chu and Zhong, 1994); *J. davurica* (Adams, Shatar and Dembitsky, 1994), *J. sabina* (Adams, Dembitsky and Shatar, 1998, Adams, 2014).

The purpose of this paper is to make the initial report on the composition of the volatile leaf oil(s) of *J. sabina* var. *balkanensis* and compare it with that of *J. s.* var. *sabina.*

MATERIALS AND METHODS

Specimens used in this study (species, popn. id., location, collection numbers):
J. sabina var. *balkanensis*

Bulgaria and Greece

BeR: Eastern Rhodopes. In protected site “Gumurdjinsky Snejnik”, locality “Madzharsky Kidik”. On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca* with *Juniperus communis*. 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m, 13 Aug. 2012, *Adams 13725-13729 (A. Tashev 2012-1-5*);

BSk: Central Stara Planina (the Balkan). National Park “Central Balkan”. Reserve “Sokolna”. On a steep, rocky limestone slope, with *Sorbus aucuparia, S. aria, S. borbasii, Amelanchier ovalis, Carpinus orientalis, Sesleria latifolia, Pastinaca hirsute, Cephalanthera rubra, Laserpitium siler, Hieracium alpicola* etc. near a forest of *Fagus sylvatica.* 42°42'13.3" N,25°08'10.4" E, 1501 m , 22.08.2015. Bulgaria, *Adams 14721 (A. Tashev 2015 Balkan 1*;

BkR: Rila Mountain, National Park “Rila”. On the eco-path, Beli Iskar”, near river Beli Iskar, in a forest with *Pinus sylvestris, P. peuce, Picea abies, Abies alba, Juniperus communis, J. sibirica, Vaccinium myrtillus, Rosa canina, Sorbus aucuparia, Acer hyrcanum, Chamaespartium sagittale, Hypericum perforatum, Thymus* sp. etc. 42°14'26.5" N, 23°32'33.8" E, 1242 m, 24.06.2015. Bulgaria, *Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2)*;

BkG: Mt. Tsena, Greece, *Adams 14727-14731* (*A. Tashev 2015 So. 1-5 Tsena*);

Turkey

Bk/Turk: Spil Daği Milli Parki (National Park), Turkey, Manisa, N38°, 57', E 27° 41', 1024 m. *Adams 14934* , (*Tuğrul Mataraci 2016-1*)

Other plants referenced in this paper:

J. chinensis, CH, Lanzhou, Gansu, China, Adams 6765-67; *J. davurica*, DV, 15 km se Ulan Bator, Mongolia, Adams 7252, 7253, 7601; *J. sabina*, SN, Sierra Nevada, Spain, Adams 7197, 7199, 7200; PY, Pyrenees Mtns., Spain/ France border, Adams 7573-77; SW, Switzerland, Adams 7611, 7612, 7614, 7615; KZ, 30 km n. of Jarkent, Kazakhstan, Adams 7811-13; AM, Altair Mtns., Mongolia, Adams 7585-88; TS, Tian Shan Mtns., Xinjiang, China, Adams 7836-38; MS, sand dunes, 80 km sw Ulan Bator, Mongolia, Adams 7254-56; AR, sand dunes, Lake Qinghai, Qinghai, China, Adams 10347-52.
Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 °C until analyzed. The extracted leaves were oven dried (100 °C, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS

The compositions of the leaf oils are given in Table 1. The leaf oils in all *J. sabina* var. *balkanensis* populations are dominated by sabinene (34.8 - 59.7%) except in the low trans-sabinyl acetate chemotypes (BeR/hiTS, 7.5%) and BkR/hiTS, 3.7%). Other moderately high components are: α-pinene (1.3 - 3.7%), myrcene (1.6 - 3.9%), γ-terpinene (0.8 - 3.2%), trans-sabinene hydrate (trace -3.1%), terpinen-4-ol (2.1 - 5.9%) and methyl citronellate (0.6 - 3.9%).

The most obvious characteristic of the *J. sabina* var. *balkanensis* oils is the presence of chemotypes in three populations. The Greece population contained a polymorphism for methyl eugenol and elemicin (both phenolics not terpenes). Two plants were hiME and 3 plants were loME. Comparing BkG/hiME vs. BkG/loME: methyl eugenol (13.2%, trace), elemicin (6.0%, trace). Interestingly, this chemotype seems restricted to these two compounds as BkG/hiME and BkG/loME oils are very similar in their other components composition (Table 1). Both chemotypes are low in trans-sabinyl acetate (Table 1).

The second chemotype is high trans-sabinyl acetate (hiTS) and low trans-sabinyl acetate (loTS) polymorphism. The eastern Rhodopes samples contained 1 hiTS and 4 loTS oils, and the Rila Mtns. samples contained 2 hiTS and 3 loTS oils. The single sample from the Sokolna Reserve, Bulgaria and the sample from Spil Dagi, western Turkey were both loTC, loME chemotype oils. Of course, with such limited sampling, one can not know the frequency of chemotypes in any of the populations sampled in this study.

The hiTS chemotype is characterized by higher concentrations of trans-sabinyl acetate (39.6, 39.8%), cis-thujone (1.7, 12.4%), trans-thujone (12.7, 6.7%), trans-sabinol (5.0, 4.3%) with lower concentrations of sabinene (7.5, 3.7%), γ-terpinene (0.8, 0.6) and terpinen-4-ol (2.1, 1.6%) (Table 1). In contrast, the loTS chemotype has lower concentrations of trans-sabinyl acetate (0.3-1.7%), cis-thujone (0 -trace%), trans-thujone (trace-0.4%), trans-sabinol (0-0.5%) with higher concentrations of sabinene (41.2-59.7%), γ-terpinene (2.2-3.2%) and terpinen-4-ol (3.9-5.9%) (Table 1).

Adams et al. (2006) reported that a Sierra Nevada, Spain population was the most polymorphic examined, with variations of: trans-thujone (0.5 to 8.7%), terpinen-4-ol (3.6- 14.4%), trans-sabinyl acetate (6.4 - 41.3%), and methyl eugenol (0.01 - 12.1%). In the Pyrenees population (PYR, Table I), citronellol was largest with 4.1%. Safrole (1.8%) was only found in PYR. Several compounds, normally associated with cedarwood oil (cedrol, α-cedrene, γ-amorphene, allo-cedrol, epi-cedrol, α-acorenol), were reported only in the Kazakhstan population (KAZ, Table I). However, Adams et al. (2006) found that cedrol (13.3%) and related compounds were also present in Tian Shan, China, *J. sabina* population. Cedrol concentrations were very variable in the *J. chinensis* samples (cedrol, 1.0 - 16.0%, Adams et al. 2006).

It is noteworthy that coahuilensol, methyl ether-coahuilensol, and pinchotene acetate (all containing the 2-ethenyl-3-methyl phenol moiety) were present only in the Bulgaria, eastern Rhodopes, loTS chemotype and *J. s.* var. *sabina*, Switzerland (Table 1). These unusual compounds were discovered (Adams et al. 2007) in *Juniperus pinchotii* and *J. arizonica*, and have been found in several taxa around the world.

The composition of the volatile leaf oils of *J. s.* var. *balkanensis* do not appear to be different in any major components from that of *J. sabina*, Switzerland. Due to the chemotypes, it is not possible to discern any geographic differentiation among the var. *balkanensis* populations sampled.

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Table 1. Comparisons of the per cent total oil for leaf essential oils for *J. sabina* var. *balkanensis* and *J. sabina* populations. *J. s.* var. *balkanensis* population codes: Bk/Turk, Turkey; BkR/loTS, Rila Mtn, Bulgaria, low trans-sabinyl acetate; BkR/hiTS, Rila Mtn, Bulgaria, high trans-sabinyl acetate; BeR/loTS, eastern Rhodopes, Bulgaria, low trans-sabinyl acetate; BeR/hiTS, eastern Rhodopes, Bulgaria, high trans-sabinyl acetate; BkG/loME, Greece, low methyl eugenol; BkG/hiME, Greece, high methyl eugenol;. *J. sabina* population codes: SN, Sierra Nevada, Spain; PYR Pyrenees Mtns.; SWZ, Switzerland; KAZ, Kazakhstan; Components that tend to separate the species are highlighted in boldface.

RI	Compound	Bk Turk	BkG hiME	BkG loME	BeR hiTS	BeR loTS	BkR hiTS	BkR loTS	SWZ	PYR	KAZ
931	α -thujene	1.2	1.2	1.7	0.5	1.3	0.3	1.0	0.9	1.0	0.6
939	α-pinene	2.4	3.7	2.6	1.3	3.0	2.2	1.3	2.0	1.8	15.8
953	camphene	t	-	t	t	t	t	t	t	t	0.3
976	sabinene	56.1	42.5	59.7	7.5	41.2	3.7	41.3	34.8	54.9	42.6
980	β-pinene	t	0.3	0.2	0.3	0.4	0.6	t	t	t	0.7
991	myrcene	3.4	2.8	3.9	2.0	2.9	1.6	2.7	4.2	3.1	3.8
1005	α-phellandrene	t	t	t	t	0.1	t	0.1	t	0.1	t
1011	δ-3-carene	0.1	1.5	t	1.0	0.1	0.1	0.1	-	0.1	0.2
1018	α-terpinene	1.2	1.4	1.6	0.4	1.6	0.3	1.6	1.0	1.5	0.7
1026	p-cymene	0.3	0.4	0.5	0.4	0.6	0.6	0.5	0.2	0.4	0.1
1031	limonene	1.5	0.8	1.3	0.6	0.6	0.8	1.0	3.0	2.4	2.1
1031	β-phellandrene	1.6	0.8	1.3	0.9	0.9	0.8	1.0	t	t	1.4
1032	1,8-cineole	t	0.1	0.1	0.2	0.4	t	t	-	t	t
1050	(E)-β-ocimene	0.9	0.3	0.2	0.1	0.5	0.1	t	1.1	0.7	0.1
1062	γ-terpinene	2.2	2.3	2.7	0.8	2.9	0.6	3.2	1.1	2.5	0.1
1068	cis-sabinene hydrate	2.3	1.6	2.1	1.1	3.2	0.5	2.3	0.7	1.4	0.5
1067	cis-linalool oxide(fura)	0.1	t	t	t	0.3	t	t	t	t	-
1088	terpinolene	1.1	1.2	1.3	0.6	1.2	0.3	1.2	0.8	1.0	0.7
1097	trans-sabinene hydrate	0.9	2.1	1.9	1.0	3.1	t	1.0	0.3	1.1	0.4
1098	linalool	1.0	t	t	1.0	3.1	0.4	1.0	1.5	0.3	0.2
1102	nonanal	-	-	t	-	-	-	-	t	-	t
1102	cis-thujone(α-thujone)	t	t	-	1.7	t	12.4	t	0.1	-	-
1114	trans-thujone(β-thujone)	0.4	t	t	12.7	0.2	6.7	0.1	0.7	0.1	-
1121	cis-p-menth-2-en-1-ol	0.5	0.4	0.4	0.2	0.5	0.2	0.5	0.2	0.6	0.2
1134	iso-3-thujanol	0.2	-	-	0.6	-	0.2	t	-	-	-
1140	**trans-sabinol**	**-**	**-**	**-**	**5.0**	**0.5**	**4.3**	**0.3**	**0.7**	**0.3**	**-**
1140	trans-p-menth-2-en-1-ol	-	0.2	0.4	-	-	-	-	-	-	0.2
1153	citronellal	0.1	t	t	-	-	-	-	0.2	0.4	-
1156	sabina ketone	0.1	t	t	-	0.2	-	0.1	-	-	-
1166	**coahuilensol**	**0.3**	**-**	**t**	**-**	**1.1**	**-**	**-**	**0.4**	**-**	**-**
1177	terpinen-4-ol	5.0	3.9	4.4	2.1	5.9	1.6	4.8	1.4	7.2	2.9
1183	p-cymen-8-ol	t	-	t	t	0.1	t	t	-	-	-
1189	α-terpineol	0.2	0.2	0.2	0.1	0.5	t	0.2	0.1	0.3	0.2
1193	cis-piperitol	0.1	t	0.2	0.1	0.4	0.2	0.3	t	0.2	0.1
1205	trans-piperitol	0.2	0.2	0.2	0.2	0.3	t	0.3	-	0.2	0.1
1219	**methyl coahuilensol**	**0.2**	**-**	**t**	**-**	**0.6**	**-**	**-**	**0.4**	**-**	**-**
1228	citronellol	0.8	0.3	0.4	0.3	2.9	t	0.2	0.6	4.1	0.4
1257	linalyl acetate	t	0.1	0.1	0.6	0.8	t	0.8	0.2	-	0.3
1261	methyl citronellate	0.3	1.0	1.6	0.6	3.3	1.2	3.9	0.7	0.8	0.1
1285	bornyl acetate	t	t	t	-	t	t	t	t	-	0.4
1285	safrole	-	-	-	-	-	-	-	-	1.8	-
1287	trans-linalyl acetate	0.2	0.6	t	0.1	0.2	t	0.1	-	-	-

RI	Compound	Bk Turk	BkG hiME	BkG loME	BeR hiTS	BeR loTS	BkR hiTS	BkR loTS	SWZ	PYR	KAZ
1290	trans-sabinyl acetate	1.7	0.5	0.3	39.6	0.4	39.8	0.4	35.0	t	-
1319	(E,E)-2,4-decadienal	t	t	t	t	0.3	0.2	t	-	-	-
1323	methyl geranate	0.4	t	0.3	t	0.4	0.9	0.4	0.3	0.1	0.1
1350	α-terpinyl acetate	0.1	t	t	0.1	0.2	t	0.2	0.1	-	0.2
1376	α-copaene	t	t	t	0.1	0.1	t	t	-	-	-
1389	2E,4E-me-decanoate	-	0.4	0.5	-	-	0.3	0.8	-	-	-
1401	methyl eugenol	-	13.2	t	0.4	0.3	4.1	5.8	-	1.1	-
1409	α-cedrene	-	-	-	-	-	-	-	-	-	0.2
1418	(E)-caryophyllene(β-caryophyllene)	t	t	0.1	0.1	t	t	0.2	-	-	-
1468	**pinchotene acetate**	**t**	**-**	**-**	**-**	**0.5**	**-**	**-**	**0.1**	**-**	**t**
1477	γ-muurolene	0.1	t	t	0.3	0.2	t	0.1	0.1	t	0.1
1480	germacrene D	0.1	t	t	0.3	t	t	t	-	0.1	-
1491	trans-murrola-4(14),5-diene	-	-	-	-	-	-	-	-	0.6	t
1493	epi-cubebol	0.1	t	t	0.3	0.2	t	0.2	0.1	0.3	-
1495	γ-amorphene	-	-	-	-	-	-	-	-	-	0.1
1499	α-muurolene	0.2	0.1	0.1	0.5	0.3	0.2	0.3	0.1	0.2	0.2
1513	γ-cadinene	0.4	0.5	0.4	1.7	0.8	0.4	0.6	0.3	0.8	0.3
1524	δ-cadinene	0.8	0.8	0.6	1.9	1.1	0.5	0.9	0.5	1.0	0.8
1538	α-cadinene	0.2		t	0.2	0.1	t	0.3	0.1	0.1	0.1
1549	elemol	0.6	t	t	0.1	t	0.5	1.9	0.8	2.1	0.1
1554	elemicin	0.1	6.0	t	0.2	t	6.4	2.3	-	0.4	-
1574	germacrene D-4-ol	2.6	1.4	1.8	2.5	2.2	0.5	2.4	1.4	0.7	1.1
1587	allo-cedrol	-	-	-	-	-	-	-	-	-	1.1
1596	cedrol	-	-	-	-	-	-	-	-	-	15.9
1606	β-oplopenone	0.7	0.7	0.3	0.4	0.6	0.5	1.1	0.1	0.3	-
1611	epi-cedrol	-	-	-	-	-	-	-	-	-	0.1
1627	1-epi-cubenol	0.1	t	t	0.2	t	0.1	0.2	t	0.4	-
1632	α-acorenol	-	-	-	-	-	-	-	-	-	0.2
1640	epi-α-cadinol	0.4	0.3	0.3	0.8	0.5	0.3	0.5	0.2	0.3	0.3
1640	epi-α-muurolol	0.4	0.4	0.3	0.8	0.5	0.3	0.6	0.2	0.2	0.3
1645	α-muurolol	0.1	0.1	t	0.3	0.2	0.1	0.2	0.1	0.1	0.1
1649	β-eudesmol	t	-	-	-	-	0.2	0.6	-	0.2	-
1652	α-eudesmol	t	-	-	-	-	t	0.6	-	0.3	-
1653	α-cadinol	1.0	0.8	0.7	1.8	1.3	0.9	1.2	0.6	0.5	0.9
1689	shyobunol	0.4	t	0.3	0.2	t	0.1	0.5	0.1	t	0.1
2054	abietatriene	t	t	t	t	t	t	t	t	t	t
2080	abietadiene	0.2	0.2	0.4	0.2	0.3	t	t	t	0.1	t
2288	4-epi-abietal	0.4	0.3	0.5	0.5	0.7	0.3	0.7	t	0.1	t
2302	abieta-7,13-dien-3-one	1.0	0.7	1.3	0.7	1.9	0.5	2.0	0.1	0.1	0.2
2325	trans-ferruginol	t	t	t	t	t	t	0.2	t	t	t
2343	4-epi-abietol	t	t	t	t	0.3	t	0.1	t	t	t

RI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Notes on the Morphology and Taxonomy of *Micromyces* (Synchytriaceae, Chytridiomycota), with special attention to *M. longispinosus*, *M. grandis*, *M. furcatus* and *M. ovalis*

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ABSTRACT

Traditionally, family Synchytriaceae has been placed in order Chytridiales, phylum Chytridiomycota. Within the family, though, the taxonomic situation has been less clear. This family has been considered to contain only a single genus (*Synchytrium*), or as many as four genera: *Synchytrium*, *Micromyces*, *Micromycopsis* and *Endodesmidium* (cf. Sparrow, 1943, 1960; Karling, 1977)--either approach perhaps tenable. However, since the 'separate genera' appear distinct, and until contravened by molecular evidence, we recognize them here. Karling (1964), while adopting the single-genus approach (recognizing only *Synchytrium*, with a number of subgenera), nonetheless provided a helpful compilation of information. However, taxonomic attention is still needed in the family. We review taxa of the aquatic genus *Micromyces* (endoparasites of conjugate algae, i.e., Zygnemataceae). Sparrow's (1960) key to species of *Micromyces* has stood well until the present. But, since Sparrow (1960) did not recognize *Micromycopsis* (including its species in *Micromyces*), and since we herein recognize *Micromycopsis*, adjustments to the *Micromyces* key were necessary. Also, certain potential species of *Micromyces* that were not included in Sparrow's taxonomic key (i.e., *M. grandis* Miller, 1955 and *M. "furcata"* Rieth, 1962) required evaluation. We determine *M. grandis* to be a large variant of *M. longispinosus* Couch (1937). *Micromyces "furcata"* Rieth (1962), subsequently accounted for mainly in listings of Chytridiomycete names, is morphologically distinct, and is added to species in our key. The epithet "furcata" (Rieth, 1962) should be "furcatus," viz. *M. furcatus* (*Index Fungorum*). *Micromyces ovalis* (Rieth, 1950) is nomenclaturally invalid (lacking the Latin diagnosis required at the time); an English diagnosis (see present rules of nomenclature) is here provided. In addition to various points of nomenclatural clarification, we hope that our observations of living material of *M. longispinosus* will add to morphological understanding of this species. Future molecular studies should inform as to relationships among *Micromyces* species, and how many genera of Synchytriaceae should be recognized. Published on-line www.phytologia.org *Phytologia 100(1): 51-61 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: host-parasite relationships, intracellular, *Mougeotia*, prosorus, resting spore, sorus, *Spirogyra*, sporangia, zoospores.

Synchytriaceous organisms are intracellular parasites of types of flowering plants, ferns, mosses, and green algae (Sparrow, 1960; Karling, 1977); one saprobic species has been reported (Longcore et al., 2016). Life cycles of taxa of Synchytriaceae are variably documented--usually by descriptive statements and line drawings--less often by photographs (helpful exceptions include Miller, 1955; Rieth, 1962; Longcore et al., 2016; and photographic illustrations, herein, of our collections of *Micromyces longispinosus*). Compared with most Chytridiomycetes, members of family Synchytriaceae have a complex life cycle (Karling, 1977), often involving formation of--not just of sporangia, but--a preceding prosorus, and a sorus (which, for a time at least, contains the sporangia). In many Synchytriaceae, a resting spore (or resting sporangium) may also form; the resting spore may function either as a prosorus or a sorus (or these latter structures can develop within, or from, the resting spore). Resting spores are, in fact, common in the family and often resemble prosori or sori; in many cases, for example, resting spores can be difficult to distinguish from prosori. In spite of unavoidable questions of structure identity, and of opposing taxonomic viewpoints, we herein recognize the four, historically recognized genera of the family: *Synchytrium*, *Micromyces*, *Micromycopsis* and *Endodesmidium*--the latter three of these genera having been, at times, submerged variously in one or more generic/subgeneric groupings, synonymies; cf.

Karling (1964, 1977), Sparrow (1960) and Dick (2001). Our dichotomous key serves to present these putative genera, emphasizing distinctions that are presently considered to be most dependable.

KEY TO THE GENERA OF SYNCHYTRIACEAE RECOGNIZED HERE

1.Occurring in conjugate algae (Zygnemataceae or Desmidiaceae); prosorus endobiotic, with a distinct discharge tube; sorus typically epibioic (at apex of discharge tube), with a definite, somewhat thickened wall which may be smooth or spiny; zoospores typically released extra-matrically....*Micromycopsis*

1.Occurring in conjugate algae, embryophytes, or rarely free-living; prosorus (if any) and sorus developmentally endobiotic; sorus sessile on or formed within the prosorus (if present); sorus with or without a distinct surrounding membrane or wall (if present, typically not thickened); zoospores released intra- or extra-matrically.

2.Parasitic in a number of flowering plants and certain mosses and ferns (there is also one reported saprobic species); young thallus not amoeboid; prosorus present or often lacking; sorus simple, frequently formed directly from (within) the thallus, relatively large (usually more than 35 µm in diameter); common soral wall or membrane present for a time at maturity…....*Synchytrium*

2.Parasitic in green algae (Conjugatae); young thallus often amoeboid; prosorus present; sorus simple or compound, relative small (usually less than 35 µm in diameter), developed externally from the prosorus and remaining sessile upon it (the two structures thus continuing for a while to be 'joined'); sorus with or without a common surrounding membrane or wall at maturity.

3.Found in members of the Zygnemataceae (e.g., *Spirogyra*, *Zygnema*, *Mougeotia*); prosorus wall frequently composed of distinct segments, often ornamented ('spiny'); sorus simple or compound, lacking a common wall or membrane at maturity (the thin wall sometimes segmenting); sporangia not amoeboid or flagellated; zoospores usually well more than 5 per sporangium…*Micromyces*

3.Found in members of the Desmidiaceae (e.g., *Netrium*, *Cylindrocystis*); prosorus wall uniform, smooth; sorus simple, wall or membrane persisting, developing two, opposite papillae; sporangia amoeboid or allegedly even flagellated; zoospores 5 or fewer per sporangium…..*Endodesmidium*

SELECTED ASPECTS OF GENERIC HISTORY OF FAMILY SYNCHYTRIACEAE

There is no need here to comprehensively review the history of this large family of Chytridiomycetes; yet, some aspects of this history are pertinent to discussion. Sparrow (1943) recognized three genera of Synchytriaceae: *Synchytrium*, *Micromyces*, and *Micromycopsis*. Sparrow (1960) still recognized three genera, but only two were the same as in 1943: Sparrow (1960) recognized *Synchytrium* and *Micromyces*, but considered *Micromycopsis* to be encompassed within a broadly defined *Micromyces* (a viewpoint adopted by Dick, 2001); Sparrow (1960), though, additionally recognized *Endodesmidium* (Canter, 1949)--according to Canter, a relatively simple, putatively primitive genus (but see Karling, 1954, 1977, for another viewpoint on primitive vs. advanced genera/species within the Synchytriaceae). Couch (1931), while emphasizing resemblance in morphology and life cycle between *Micromyces* and *Synchytrium*, nonetheless retained these genera. Karling (1953, p. 278), however, merged *Micromyces* and *Synchytrium*, based on "fundamental structural and developmental similarities," transferring species of *Micromyces* to *Synchytrium.* Karling (1964) subsequently enumerated six or seven (cf. p. 118 vs. pp. 114-115) subgenera within *Synchytrium* (some subgenera traceable to earlier authors, e.g., Fitzpatrick, 1930); subgenus *Microsynchytrium* housed, among other species, species previously classified in *Micromyces.* In Karling (1964), all genera and species of Synchytriaceae were subsumed under *Synchytrium.* Enigmatically, Karling (1977)--retaining the subgenera of *Synchytrium*, as *de facto* an additional presentation within his overall coverage--resurfaced recognition (perhaps for pragmatic reasons) of the four, classic genera of Synchytriaceae (*Synchytrium*, *Micromyces*, *Micromycopsis* and *Endodesmidium*). The question of genera vs. subgenera, within the Synchytriaceae, has remained unsettled. But, because it has *not* been shown through molecular analysis that aquatic genera such as *Micromyces* and *Endodesmidium* should be merged with the terrestrial *Synchytrium*, there is still no definitive argument against recognizing the four 'traditional' genera of Synchytriaceae--including *Micromycopsis*--which seem morphologically and adaptively distinct (see generic key, above). It bears mention that several other alleged genera of Synchytriaceae have been named; however, these are generally considered synonyms of *Synchytrium* (see Karling 1977, p. 50). *Johnkarlingia* Singh & Pavgi--a parasite of roots of cauliflower and cabbage--is listed as a genus of Synchytriaceae in *Index Fungorum* (IF), but Karling (1977) included this ('intermediate') genus under coverage of Olpidiaceae.

MORPHOLOGY AND LIFE CYCLE OF *MICROMYCES LONGISPINOSUS* (Figs. 1-6, 11-22)

Micromyces longispinosus, described by Couch (1937)--based on earlier observations of what Couch (1931) initially thought was a collection of *M. zygogonii*--occurs in vegetative cells of *Spirogyra* and *Mougeotia* (see Sparrow, 1960), often in the vicinity of the nucleus. The young thallus develops into a generally spherical, hyaline to yellowish or brownish, uninucleate prosorus (10-33.6 µm in diameter, not counting the spines) which typically exhibits 12-24, straight or curved, transparent, slender, gradually tapered spines which can be at least 22 µm in length (averaging ca. 12 µm, and usually exceeding 9 µm). The often irregularly reticulate prosoral wall or membrane can eventually become partitioned into several or a number of segments (each bearing one or more spines), as can the thinner-walled sorus. The sorus develops from the prosorus, is spherical, smooth-walled or somewhat spiny, and sessile upon the prosorus; nuclear divisions typically take place in the sorus (pursuant to migration of the prosoral nucleus into the sorus). The soral membrane disappears at maturity (or its segments become incorporated in sporangial walls/membranes)--revealing 8 to 24 (possibly more), thin-walled sporangia, which are not flagellated or amoeboid. The rounded to somewhat elongate (sub-conical), sometimes angular sporangia--typically 8 and 10 µm in size--can each release (through a pore in the narrowed apex) numerous, minute (ca 1 µm each), posteriorly uniflagellate zoospores--often inside the algal host-cell which, in response to parasitism, may or may not exhibit hypertrophy (when present, similar to that caused by *M. zygogonii*; see Sparrow, 1960, pp. 195, 197). The sub-spheroid to ovoid zoospores typically manifest the lipoid globule (or two globules, oppositely placed) characteristic of chytrid zoospores; zoospores *may* fuse, in pairs, apparently functioning as gametes (further confirmation desirable, cf. Karling, 1977, p. 50). Resting spores brownish, resembling prosori but averaging smaller (16-21 µm) and developing a more thickened wall, functioning as prosori (Fig. 21) or directly as sori (Fig. 19). Photographs from collections WB293 (Northport Trestle, temporary ponds) and WB294 (Marrs Spring), environs of Tuscaloosa, AL.

MICROMYCES GRANDIS (Figs. 7A,B), COMPARED WITH *M. LONGISPINOSUS*

Miller (1955) described *M. grandis* as a new species of *Micromyces*. *Micromyces grandis* was noted by Sparrow (1960) but--perhaps due to a publication date close in time to the preparation of his monumental manuscript--not included in his *Micromyces* species key. *Micromyces grandis*, occurring in a large *Spirogyra* (not named to species), is very similar to *M. longispinosus*, except for (Miller, 1955, p. 254) its "large size in all phases of its life cycle," and [causing the] "unique hypertrophy of the host cell resulting in peculiar geniculations [enlarged bends]." Miller precisely described and illustrated this 'new species,' including its thallus (which becomes the prosorus) and an association between the developing prosorus and the nucleus of the *Spirogyra* host--a phenomenon also observed in *M. longispinosus* (see our Figs. 1-4, and 14)--invoking interesting questions as to the details of host-parasite relationships. In any event, the problem, taxonomically, is the striking similarity of *M. grandis* to *M. longispinosus*, in almost all characters, including potential causation of hypertrophy of the host cell (see our Fig. 12 of *M. longispinosus*). Palpable differences lie in size comparisons, *M. grandis* averaging larger than *M.*

longispinosus. But even as regards size, there is some character overlap. The prosorus of *M. grandis* ranges, according to Miller, from 30.4 to 51.2 µm; however, the prosorus of *M. longispinosus* may exceed 31 µm. Lengths of prosoral spines of *M. grandis* are, as indicated by Miller, longer than (some perhaps twice the length of) those of *M. longispinosus*; but, in this case, one is merely comparing long spines with, morphologically very similar, longer spines. Resting spores overlap in diameter, and zoospores of *M. grandis* average only about 1 µm larger than *M. longispinosus*. Zoosporangial measurements are larger in *M. grandis*, yet verge on intergradation with *M. longispinosus*. Though *M. grandis* is on the whole larger, it is otherwise essentially identical to *M. longispinosus*, and does not seem to warrant species distinction; *M. grandis* should probably be considered a large example of *M. longispinosus*. Size differences of *M. grandis* (compared to *M. longispinosus*) may have as much to do with its occurrence in a large *Spirogyra* host as with its own 'unique' charactereristics. We note in passing that Karling (1964, p. 124) transferred *M. grandis* to *Synchytrium* as *S. grandis* (*S.* "*grande*," cf. Dick 2001, p. 427), a combination pertaining only if *Micromyces* is considered part of *Synchytrium* (not presently defensible).

THE STATUS OF *MICROMYCES FURCATUS* (Figs. 8 and 9)

Compared with other species of *Micromyces*, *M.* "*furcata*" (Rieth, 1962) has been largely overlooked; the timing of publication (1962) in part accounts for this. This species was described after Sparrow's (1960) revised treatise of "Aquatic Phycomycetes." It is also not in Karling's (1964) account of the Synchytriaceae (perhaps 'in press' when *M. 'furcata'* became known). Karling's (1977) coverage of Chytridiomycetes contains a brief reference to this taxon--in a figure legend (his fig. 31, plate 16); Karling noted in this legend: "Resting prosorus of *S. furcata* (Rieth) comb. nov., with reticulate outer wall bearing dichotomously branched spines (Rieth, 1962.)." Karling (1977) was thus attempting transfer of *Micromyces* "*furcata*" to genus *Synchytrium*. But even if one accepts this taxon in *Synchytrium* [We believe it should be retained, presently, in *Micromyces*], Karling possibly did not provide enough nomenclatural information to validate a new combination (Article 33, *International Code*). Longcore's (1996) enumeration of Chytriodiomycete names (since 1960) included *M.* "*furcata*" Rieth (1962) under *Micromyces*. *Index Fungorum* (IF) lists this taxon under *Micromyces* (not *Synchytrium*), adjusting the epithet spelling (correctly) to *M.* "*furcatus*." Hence, after Rieth (1962), other than Karling's (1977) 'transfer,' *M. furcatus* appears in two itemizations of chytrid names (Longcore, 1996; and IF, present), without further information. It was not listed by Dick (2001, pp. 429-431) among taxa of Synchytriaceae.

Rieth (1962) set forth his original description of *Micromyces* "*furcata*" in a relatively obscure supplement to the publication *Die Kulturpflanze*--unintentionally contributing to this taxon being, initially, poorly known. Nonetheless, Rieth's descriptive presentation and illustrations are clear. Even though released zoospores were not observed, there is no question of the identity of the organism as *Micromyces*, or its distinction as a species. Rieth, writing in German, provided a Latin description--validating the species name (*M. furcata*). He also provided an English description (p. 294-295), quoted below (with adjustments for current usage, e.g., µm for µ) since the reference proved hard to obtain:

"Prosorus spherical, 15.5-21 µm in diameter (23-28.5 µm including spines), outer wall regularly reticulate, bearing dichotomously branched, hyaline spines. Sorus emerging through a circular pore formed in the wall of the prosorus, at first spherical, 16.5-22 µm in diameter, internally dividing in 8-16 sporangia with rather thick uncolored external wall (correspondent with the wall of the sorus). Liberation of the zoospores not seen. Resting spores spherical (7.5-)16.5(-22) µm in diameter (without the spines), outer wall regularly reticulate, covered with hyaline, dichotomously branched spines (1-)3.5(-9) µm long." [For photographs, see Rieth, 1962, Taf. II] "Parasitic in *Spirogyra longata*... Germany."

As Rieth (1962) noted, *M.* "*furcata*" [= *furcatus*] is distinguished (from other species of *Micromyces*) by *branched* spines (Figs. 8,9), on the surface of the prosorus and resting spore. These spines are mostly (distally) dichotomously branched, but may be trichotomously or otherwise branched, rarely simple (as per Rieth's original figs. A-F, "Abb. 4"); the spines average shorter than those of *M. longispinosus* (see our account of *longispinosus*). Rieth considered that the regularly reticulate wall, of the prosorus and resting spore (Fig. 9) of *M. furcatus*, was distinctive; however, similar if somewhat more irregular reticulation or segmentation may occur in *M. longispinosus* (cf. Couch, 1937; Sparrow, 1960). Spines of *M. furcatus* (cf. Rieth, 1962) appear more regularly arranged (somewhat more helically seriate) than the scattered spines of *M. longispinosus*--see Couch, 1937--which can themselves though sometimes be in local radial or polygonal arrangements. The relatively thin, soral wall-segments of *M. furcatus* (as perhaps in some other species of *Micromyces*) may become incorporated in, or attached to, portions of the walls of sporangia (Fig. 8). In any event, we believe (especially based on the unique, distinctly branched spines of both the prosorus and the resting spore) there is sufficient evidence to recognize *M. furcatus* Rieth (1962) as a distinct species of *Micromyces*, and we include it here in our key to species.

TAXONOMY AND NOMENCLATURE OF *MICROMYCES OVALIS* (Fig. 10)

Rieth (1950, Österr. Bot. Z., 97: 516) described a new species of *Micromyces*, *M. ovalis* (included and accepted by Sparrow, 1960). This species is distinguished (see Fig. 10) by separated or spaced circles of spines (each spine shark-toothed-shaped) around the perimeter of an ellipsoid prosorus, and formation of four (usually four only), tetrahedrally placed sporangia within the sorus--the soral wall not always completely tearing open. Rieth's (1950) description and illustrations are definitive; he provided his description in German, but not, however, in Latin--leaving this name (*M. ovalis*) invalid by nomenclature pertaining at that time. As permitted by current rules of nomenclature, the name is *here validated* by *English translation* of Rieth's original (German) description [Rieth did not give an account in English]:

DESCRIPTION (translated from Rieth, 1950, p. 516): Prosorus ellipsoid, 12 to 18 µm long, 8 µm wide; outer wall with 4 to 6 rings or circles of shark's-tooth-shaped spines (these apparent circles sometimes preceded by a more spiral arrangement of the developing spines). Sorus spherical or nearly so [perhaps eventually becoming somewhat distended], developing four (tetrahedrally disposed) sporangia within; these sporangia, as a rule, not completely separating from one another, the inner walls of the sporangia tending to remain connected at a mutual point; the sometimes lightly granulated sorus-wall may tear open around the tangent [outer surface] of each sporangium. Swarmers [zoospores] numerous, essentially spherical, 1 µm in diameter (each with a single, backwardly directed flagellum), escaping the sporangium (into the host cell) by one or two pores in the sporangial wall, their further destiny unknown. Resting cells [resting spores] probably prosorus-like [re: appearance and probable function].

TYPIFICATION: As indicated by Dick (2001), the type locality of *Micromyces ovalis*, originally described by Rieth (1950), is near Tübingen, Germany ('woods-pond' at Spitzberg; found parasitizing *Mougeotia* sp.). Dick noted that type material was "not verified;" actually, it appears not to have been designated. Hence, we here designate Rieth's Fig. ("Abb.") 3; *in* Österr. Bot. Z., 97: 511 (1950)--showing prosorus, sorus, and developing sporangia--as Holotypus of *M. ovalis* (see our Fig. 10 for a similar depiction). ETYMOLOGY: The epithet, *ovalis*, refers to the ovoid [ellipsoid] form of the prosorus.

OTHER COLLECTION: See discussion by Sparrow and Barr (1955) as concerns a collection of *M. ovalis* (their var. *giganteus*; cf. Literature Cited, and our Species Key below), parasitic in *Zygnema*, from Smith's Bog, Cheboygan Co., Michigan, in 1954. Sparrow and Barr (1955) incorrectly reported Rieth's (1950) original collection of 'typical' *M. ovalis* as being from Austria, rather than Germany.

CITATION OF AUTHORSHIP: Based on presentation, above, of an English translation of Rieth's (1950) initial German description (and designation here of the Type)--thus validating Rieth's species name--a more complete citation of authorship, from this point, is: *Micromyces ovalis* Rieth ex W. H. Blackw., in Blackwell, Letcher & Powell (this publication).

GENE SEQUENCE, ZOOSPORE ULTRASTRUCTURE: No *Micromyces* species has yet been sequenced or examined ultrastructurally. MYCOBANK # for *M. ovalis* = 301031 (preexisting).

UPDATED KEY TO SPECIES OF GENUS *MICROMYCES* Dangeard (1889)

(Questionable species, cf. Sparrow, 1960, or those transferred to *Micromycopsis*, not included)

1.Prosorus wall smooth…………………………………………………..…*M. laevis* Canter, 1949

1.Prosorus wall ornamented (typically 'spiny,' in one form or another).

2.Spines of prosorus distinctly (often dichotomously) forked…........*M. furcatus* Rieth, 1962*

2.Spines of prosorus simple.

3.Prosoral spines (9-)12 to 22 µm in length (sometimes exceeding 40 µm in the '*grandis*' form), slender, straight or generally curved, gradually tapered..*M. longispinosus* Couch, 1937**

3.Prosoral spines typically less than 8 µm long (rarely as much as 12 µm), narrowly to broadly conical, straight or somewhat curved (the tip gradually or abruptly attenuating).

4.Spines of prosorus relatively few, scattered, short-conical or sometimes more elongate; zoospores spindle-shaped, averaging 6 µm by 2 µm, sometimes amoeboid...*M. petersenii* Scherffel, 1926

4.Spines of prosorus more numerous, usually covering the surface, elongate-conical; or shorter and occurring in distinct, separated circles; zoospores spheroidal, 1-2 µm in diameter, not amoeboid.

5.Prosorus spherical, the spines narrowly conical, numerous, generally covering the prosoral surface...*M. zygogonii* Dangeard, 1889***

5.Prosorus ellipsoidal, the spines coarser (shaped like shark's teeth), occurring in 4 to 6, distinct, spaced rings……………..…………………*M. ovalis*, see Rieth, 1950****

*Not included in previous keys.

**Interpreted here to include *M. grandis* Miller (1955), apparently a larger variant.

****M. zygogonii* is the Type of *Micromyces*. *Micromyces bulbosus* Kadłubowska (1999), considered a nomenclaturally invalid name by IF, is in any case likely assignable to *M. zygogonii*. Sparrow (1960) mentioned *Micromyces mesocarpi* De Wildeman (1900) under discussion of *Micromyces zygogonii*; however, in light of the "*mince canal*" (slender discharge tube?) to the exterior of the algal host (see Wildeman, p. 1), *M. mesocarpi* probably belongs in genus *Micromycopsis*.

****A variety, *M. ovalis* var. *giganteus*, was described by Sparrow and Barr (1955), which would nonetheless still key out at the same point (above) as 'typical' *M. ovalis*; Sparrow (1960) noted that Rieth (1956) considered the 'prosorus,' indicated (by Sparrow and Barr) for this 'new variety,' to most likely represent merely a resting spore stage [of typical *M. ovalis*].

MOLECULAR CONSIDERATIONS

Of the four genera we recognize (see generic key), the only taxa of Synchytriaceae that have apparently been sequenced (18S, i.e. SSU, rDNA)--see Smith et al., 2014; and GenBank--are species that would be placed (cf. various classification: Sparrow, 1943, 1960; Karling 1977; Dick, 2001) strictly in the terrestrial genus *Synchytrium*--including the recently described, saprobic species, *S. microbalum* (Longcore et al., 2016). DNA sequence information is seemingly still unavailable for aquatic members of the Synchytriaceae, i.e., species traditionally placed in *Micromyces*, *Micromycopsis*, or *Endodesmidium*. We note that Karling (1964) considered species of *Micromyces* to be in subgenus *Microsynchytrium* of genus *Synchytrium*, and we note that Smith et al. (2014) included several species from *Microsynchytrium* in their analysis. However, the species of subg. *Microsynchytrium* included in Smith et al. (2014) were terrestrial--no aquatic species (as might be placed in *Micromyces*) were included. Until putative genera other than *Synchytrium* (*sensu stricto*) can be included in molecular studies of Synchytriaceae, the traditional taxonomy of the group (recognizing four genera, based on morphology; see our generic key) should be retained--i.e., there is presently insufficient evidence to argue against this viewpoint; this conclusion is drawn in spite of Karling's (1964) suggested, admittedly useful, sub-generic breakdowns of *Synchytrium* (in which he recognized no genus of Synchytriaceae other than *Synchytrium*). It is already apparent, though, from molecular analyses (Smith et al., 2014; Longcore et al., 2016) that some subgenera of *Synchytrium*, recognized by Karling (1964), are polyphyletic (including subgenus *Microsynchytrium*).

ACKNOWLEDGEMENTS

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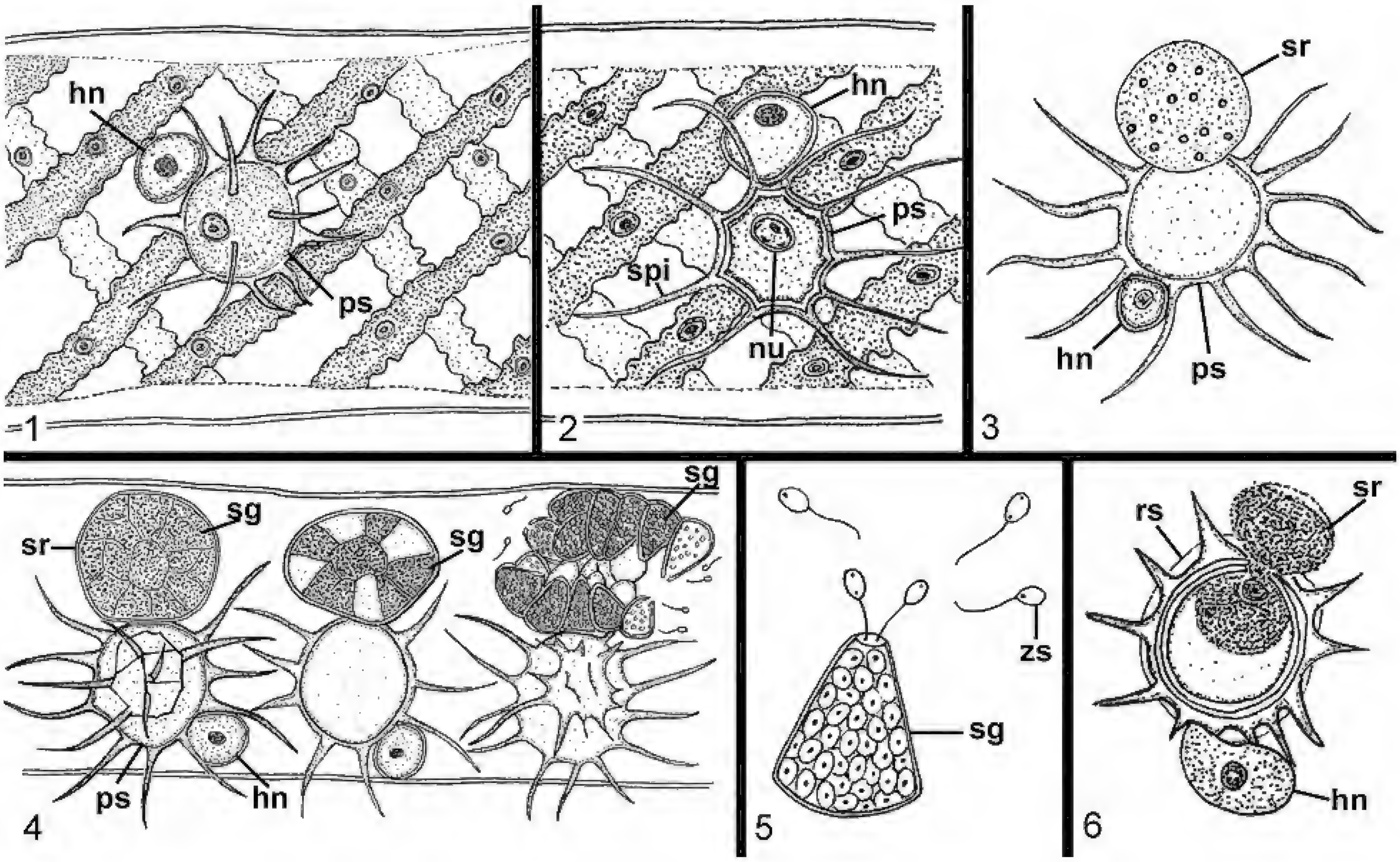

Figures 1-6. *Micromyces longispinosus.* **Fig. 1**: Developing prosorus (ps) in host cell (*Spirogyra*); note association of prosorus with host nucleus (hn). **Fig. 2**: Older prosorus (ps) continues to have a single nucleus (nu) and also remains in association with the host nucleus (hn); spines (spi) of the prosorus have become more elongate. **Fig. 3**: Germinated prosorus (ps); sorus (sr) developing from and sessile upon the prosorus, the sorus becoming mutltinucleate; host nucleus (hn) remaining in association with prosorus. **Fig. 4**: Prosorus (ps), sorus (sr) and host nucleus (hn) still associated; sorus (sr)--left to right--developing sporangia (sg) within, the sporangia slowly separating (right) as the soral membrane has broken down. **Fig. 5**: Sporangium (sg) releasing uniflagellate, ovoid zoospores (zs) from its apex. **Fig. 6**: Thickened-walled, spiny resting spore (rs) functioning as a prosorus and germinating to form a sorus (sr); host nucleus (hn) seen in association with the resting spore. Fig. 1 after Couch 1931, Sparrow 1943. Fig. 2 after Couch 1937, Karling 1964. Fig. 3 after Couch 1931, 1937; Karling 1964. Fig. 4 after Couch 1931, 1937. Fig. 5 after Couch 1937, Karling 1977. Fig. 6 after Couch 1931, 1937.

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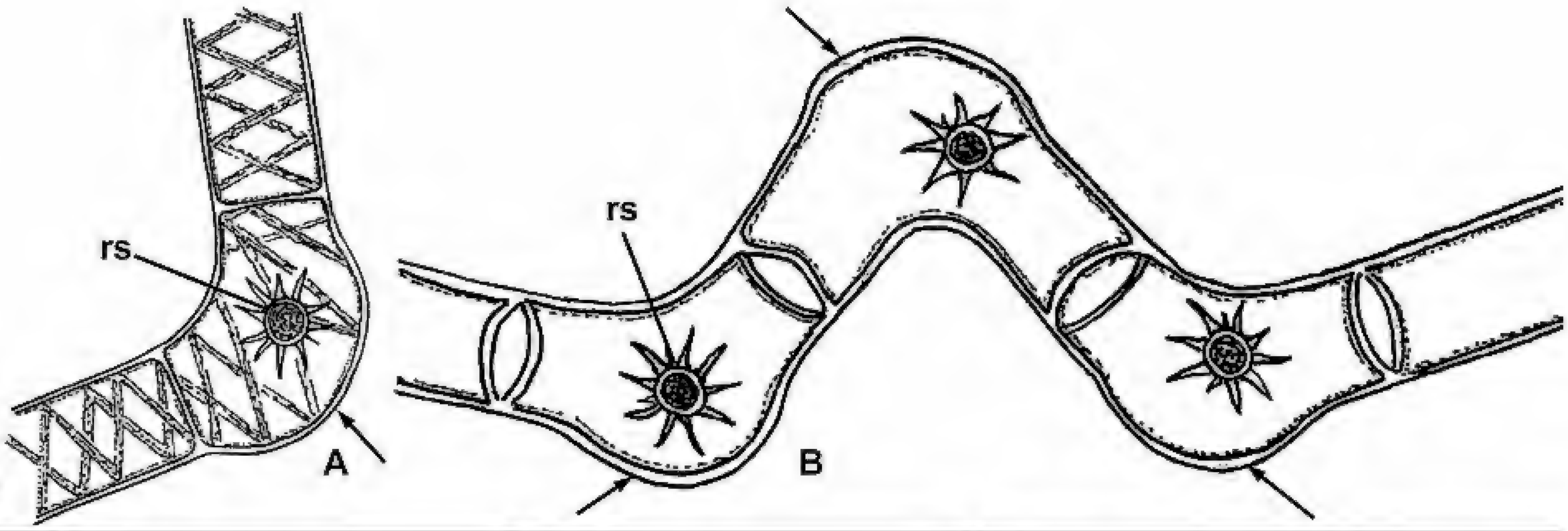

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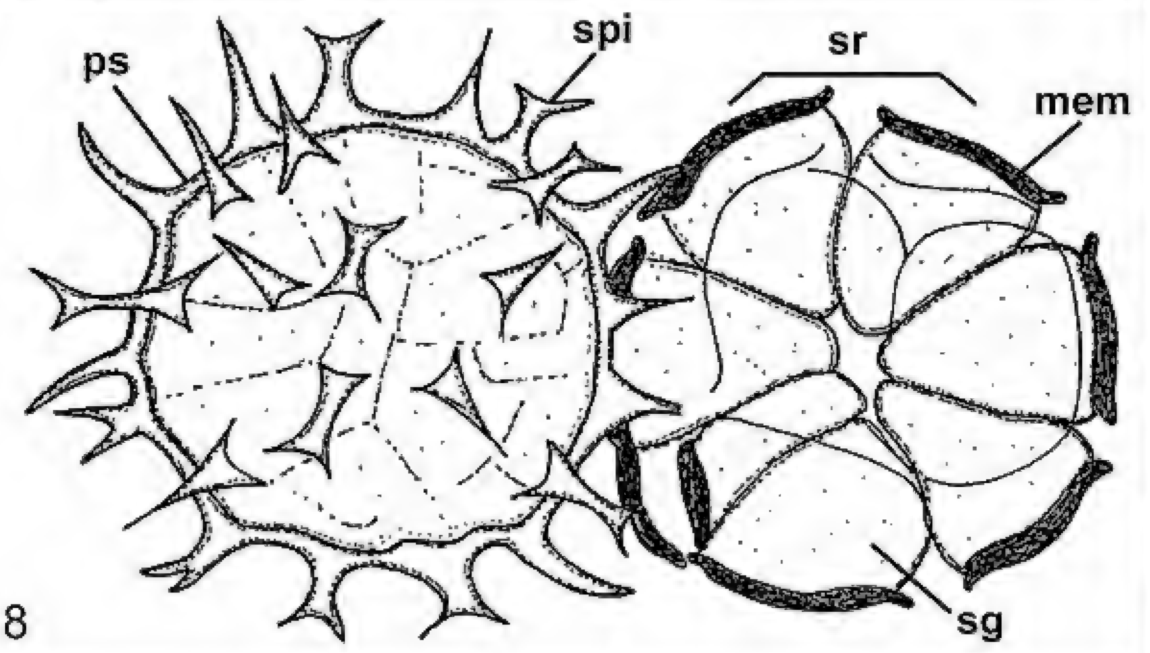

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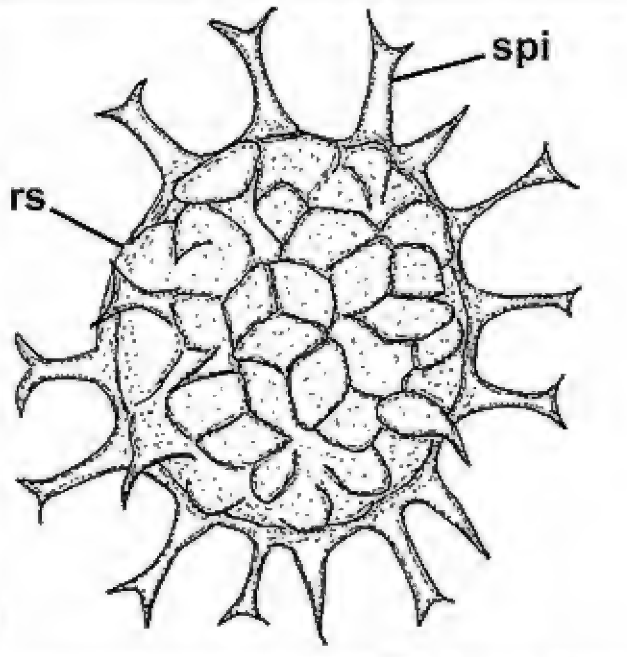

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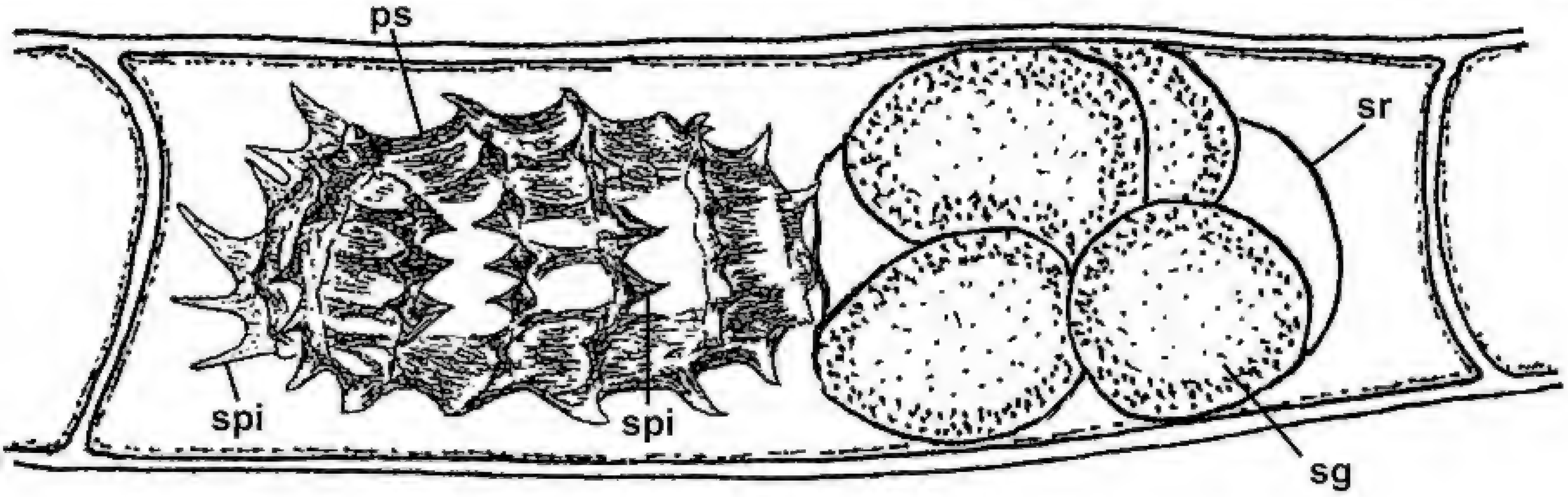
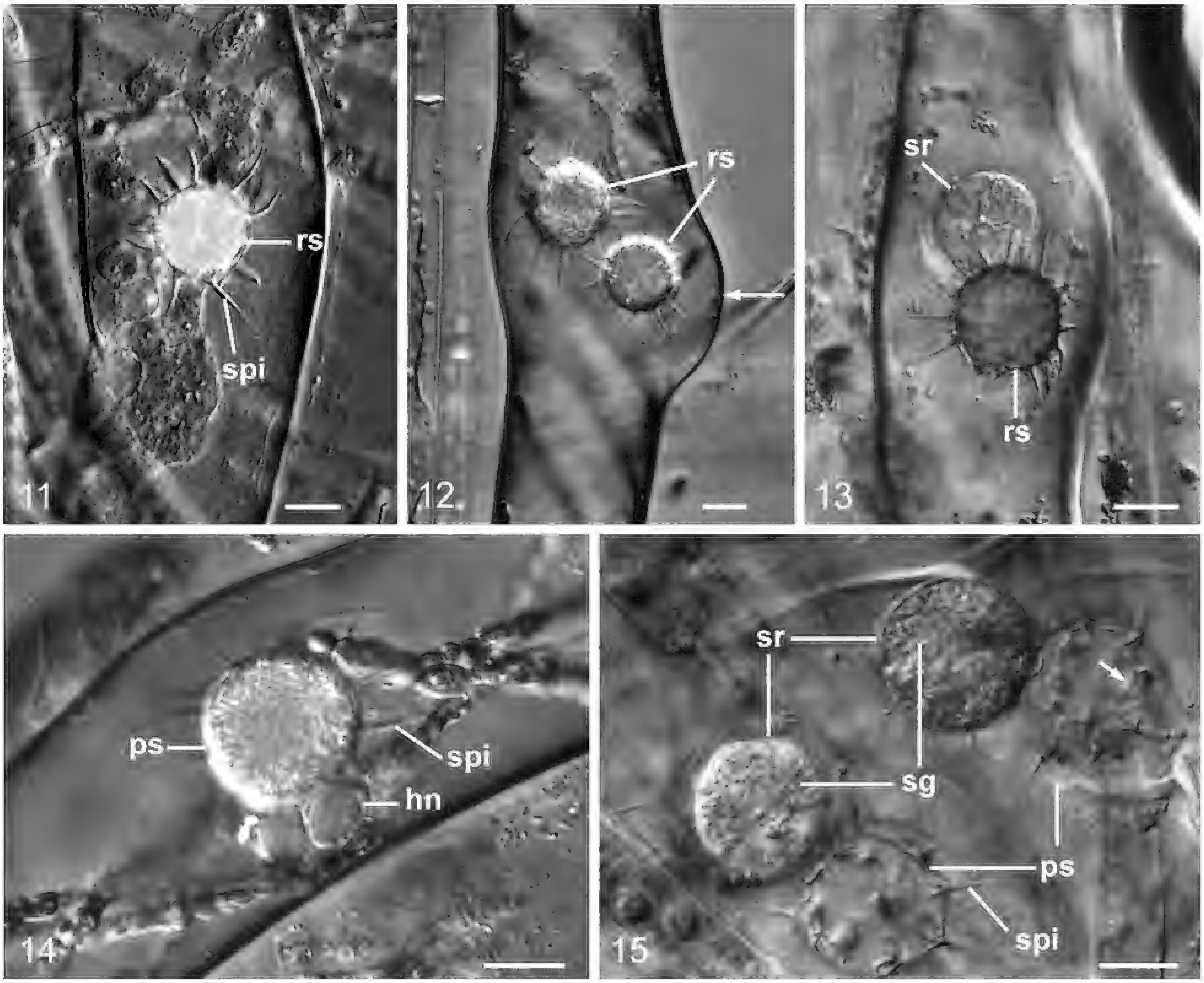
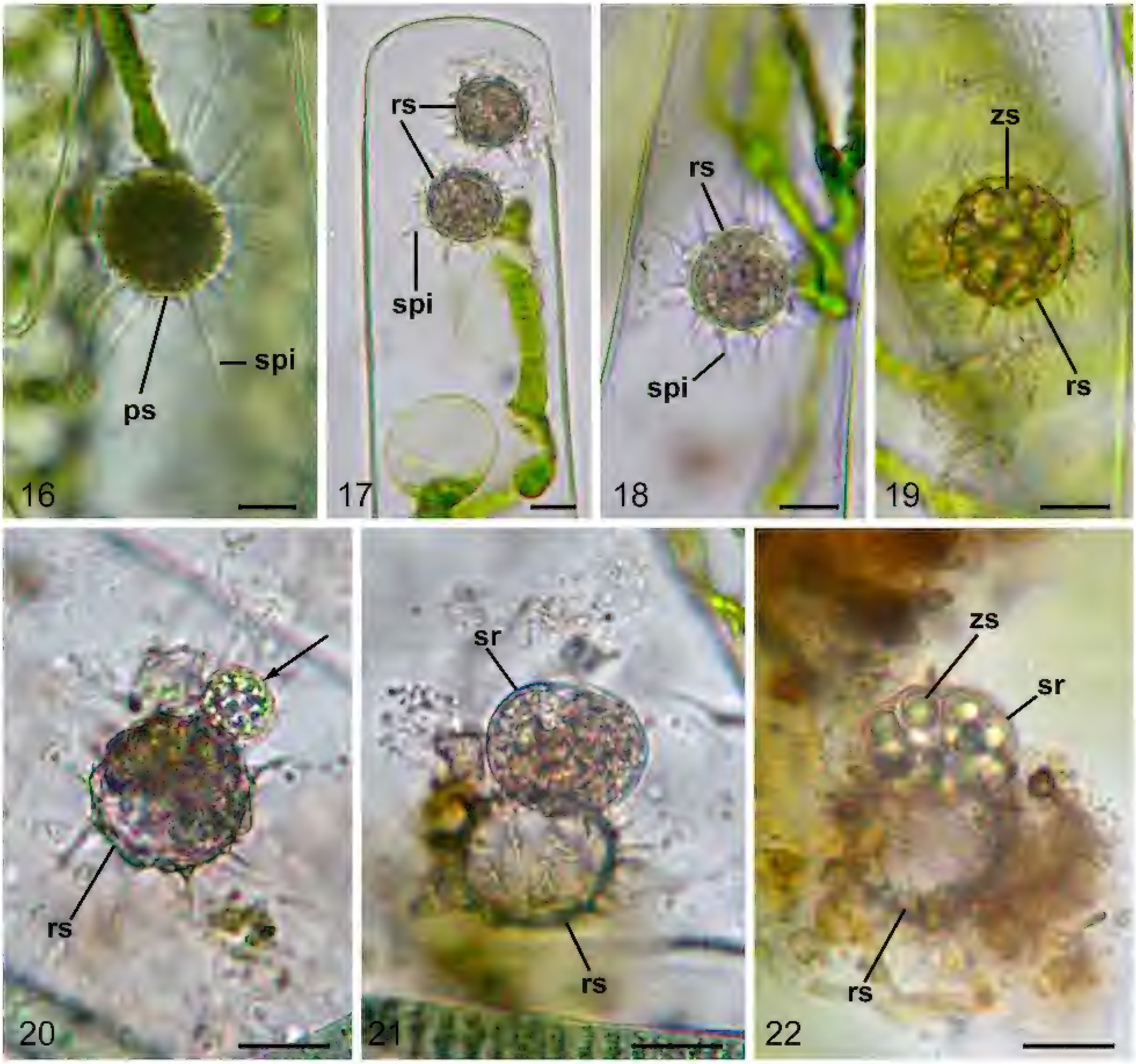


Figure 7 (A,B): *Micromyces grandis*--seemingly a large variant of *M. longispinosus*--in *Spirogyra* host. Resting spores (rs) of '*M. grandis*' in association with hypertrophy (bends, bulges--arrows) of host cells. **Figures 8-9**: *Micromyces furcatus*. **Fig. 8**: Prosorus (ps) with *forked* spines (spi); sorus (sr) containing sporangia (sg); segments of soral membrane (mem) contribute to 'outer walls' of sporangia. **Fig. 9**: Resting spore (rs) showing forked spines (spi) and reticulate wall surface. **Figure 10**: *Micromyces ovalis*: Ellipsoid prosorus (ps) with separated rings of tooth-like spines (spi); distended sorus (sr) with four sporangia (sg). Fig. 7 generally after Miller 1955 and descriptive information in Sparrow 1960. Figs. 8-9 after Rieth 1962, Karling 1977. Fig. 10 after Rieth 1950, Karling 1964.



Figures 11-15. Black & White (Nomarski) photomicrographs of *Micromyces longispinosus* in *Spirogyra* host. **Fig. 11**: Developing resting spore (rs); note distinct spines (spi). **Fig. 12**: Two young resting spores (rs); note bulge (hypertrophy) of host cell (arrow). **Fig. 13**: Resting spore (rs) functioning as a prosorus and producing an external sorus (sr) sessile upon it. **Fig. 14**: Relatively young prosorus (ps); note scattered spines (spi) and association of host nucleus (hn) with prosorus. **Fig. 15**: Two prosori (ps) which have each produced a sorus (sr); note spines (spi) and some evidence of reticulation (arrow) on prosoral wall; note early cleavage, into sporangia (sg), within the sori. Scale bar = 10 μm.



Figures 16-22. Color photomicrographs, *Micromyces longispinosus* in *Spirogyra* host. **Fig. 16**: Prosorus (ps) with slender, elongate spines (spi). **Figs. 17-18**: Resting spores (rs); note spines (spi) somewhat shorter and 'stouter' than those of prosorus. **Fig. 19**: Resting spore (rs) functioning (internally) as a sorus (forming groups of zoospores, zs). **Fig. 20**: Resting spore (rs) early germination stage (arrow). **Fig. 21**: External sorus (sr) developed from resting spore (rs) functioning as a prosorus. **Fig. 22**: Zoospores (zs) cleaved within external sorus (sr) developed from resting spore (rs). Scale bar = 10 µm.

Geographic variation in natural rubber yields in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers)

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ABSTRACT

Populations of *Helianthus annuus,* ranging from eastern Oklahoma to coastal southern California, were sampled and the yields of natural rubber (NR) from leaves determined by Accelerated Solvent Extraction (ASE). The highest yielding populations were Mill Creek, UT with a yield of 20.8 mg/ g and in the Waco, TX area with 14-18.2 mg/ g leaf DW. The lowest yields were in Preston, ID (1 mg/g), Redmond, OR (2 mg /g), Eagle Lake, NM (2.7 g/ mg), Post, TX (3 mg/g) and Quanah, TX (3 mg/ g). The highest yielding individuals were found in Mill Creek, UT (42 mg /g) and McLennan Co., Waco, TX (31 mg/ g). The appears to be no geographical relationship to the rubber yields. The highest yielding populations are in very different eco-systems with different soils, climates and ecologies. Adjacent populations are often quite different in the concentration of rubber. The most variable populations were San Diego, CA (0 - 16 mg/ g), Logan, UT - Preston, ID (0 - 12 mg/g), Dodge City, KS (0, 0, 7,8,10 mg/ g), and Meade, KS (2, 2, 4, 18 mg/ g). There is some geographic trends in rubber variation in central Kansas. But, other highly variable populations are isolated in different ecosystems. Additional studies needed to more fully understand the variation. Published on-line www.phytologia.org *Phytologia 100(1): 62-70 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, natural rubber, geographic variation.

Stipanovic et al. (1980) reported on rubber content from 23 wild Helianthus species, 2 commercial varieties and one wild hybrid, grown at the USDA Bushland, TX garden. The rubber yields from these taxa varied from 16.7 mg /g (DW leaves) in *H. agrestis* to 1 mg/ g in *H. neglectus.*

Adams and Seiler (1984) expanded the Stipanovic et al. (1980) study by examining 39 taxa of sunflowers, grown in the garden at USDA Bushland, TX), for their rubber, cyclohexane (hydrocarbon), methanol (resins) and protein concentrations. They reported that rubber varied from 17.8 mg/ g (*H. californicus*, Napa, CA and *H. resinosus*, Collins, MS to 1 mg/ g (*H. neglectus*, Kermit, TX). Seiler, Carr and Bagby (1991) analyzed 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. Rubber was found to be of lower molecular weight than *Hevea* rubber, but still appeared to be useful as a plasticizing additive and for coatings inside pipes and containers.

Recently, a new method was developed for the solvent extraction of rubber from *H. annuus* (Pearson et al. 2010a) using an Accelerated Solvent Extraction (ASE) machine. Yields of natural rubber for *H. annuus* leaves ranged from 9 mg/ g to 17 mg/ g rubber in sunflower cultivars grown under field conditions (see Fig. 4, Pearson et al. 2010b). ASE was found to be of considerable use in quickly screening numerous plants for rubber (and resins, etc.). There appear to be no publications concerning the geographical variation in the yields of rubber from *H. annuus*. ASE technology provided us an opportunity to screen large numbers of plants for rubber content.

Adams, et al. (2017a) screened natural populations of *H. annuus* for pentane extractable hydrocarbons and found the highest yielding populations in the Texas panhandle, where the plants were stressed by wind, insect and disease damage (Fig. 1). Plants from these high yielding populations returned to typical (lower) yields when grown under lush conditions in a greenhouse (Adams, et al. 2017b).

The lowest yields of hydrocarbons were found in AZ and NM and throughout the southwest (Fig. 1). Notice (Fig. 1) the WO (Woodward, OK) population had a very low yield (2.6%). The leaves were glabrous, suggesting the WO site is of hybrid origin from locally cultivated sunflowers.

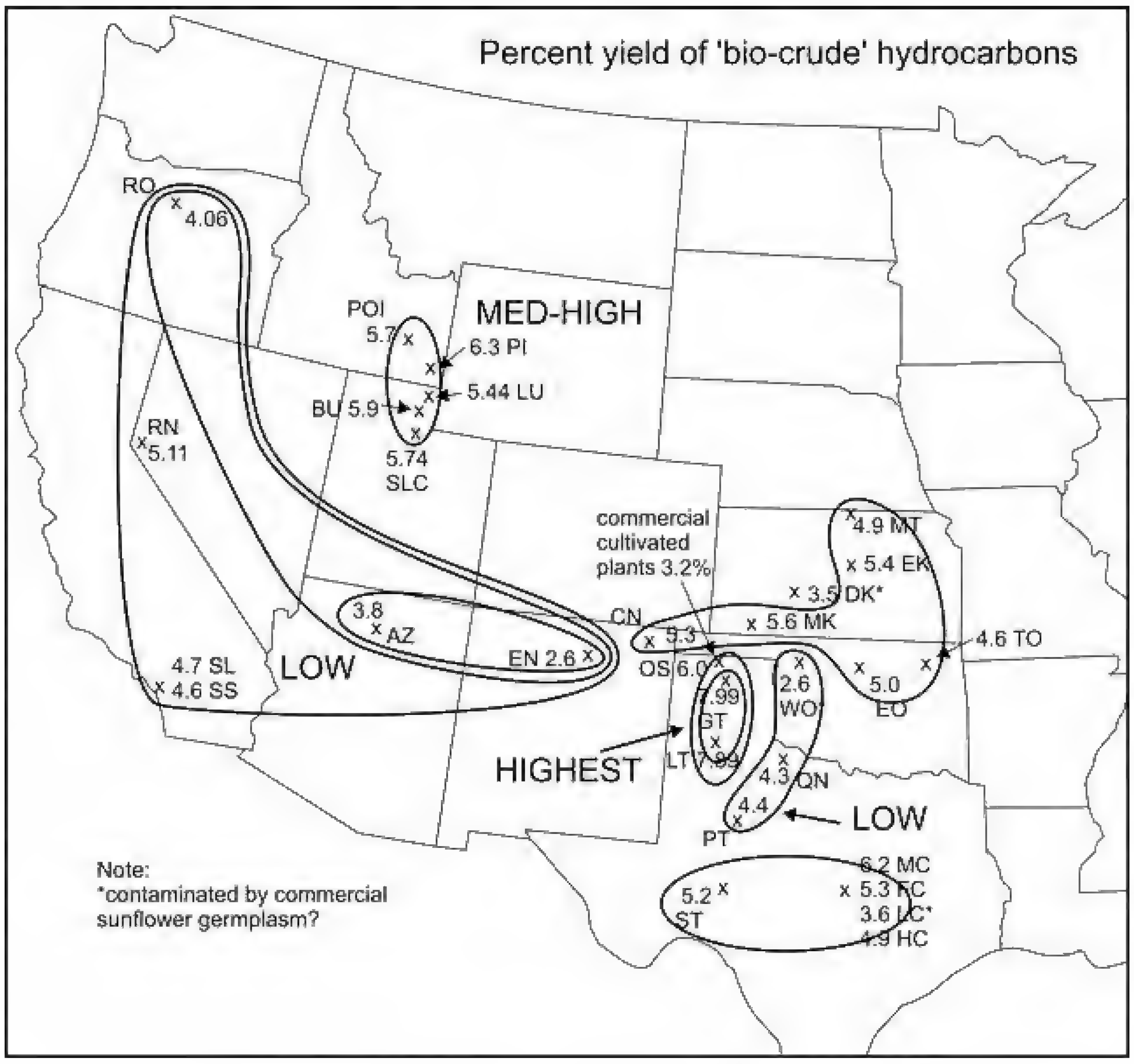

Figure 1. Geographic variation in % yields of HC by population. The asterisk (*) at the WO population indicates that the population is likely of hybrid origin between native and cultivated sunflowers. Note the low yield (3.2%) from commercial sunflowers near Oslo, TX. See text for discussion.

Our large collection of air-died leaves of *H. annuus* was found to be suitable for ASE extraction of rubber. The purpose of this report is to present new information on geographic variation of the yields of rubber in native, annual sunflower, *H. annuus*. This is a continuation of our research on sunflowers (Adams and TeBeest, 2016; Adams, et al. 2016).

MATERIALS AND METHODS

Population locations - see Appendix I.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). Leaf rubber concentration was quantified by Accelerated Solvent Extraction (ASE) using a Dionex ASE 200 (Thermo Fisher Scientific, Waltham, MA). Dried and ground samples were weighed to 0.5000g ± 0.0100g and mixed with approximately 14g of inert Ottawa sand (20-30 mesh) (Thermo Fisher Scientific, Waltham, MA) before loading into 11mL stainless steel extraction cells. The sequential three-solvent extraction protocol (Pearson et al., 2010a) started with two acetone extractions (16 minutes each) at 40°C, then five methanol extractions (5 minutes each) at 60°C, and finally two hexane extractions (16 minutes each) at 40°C. Collection vials containing the mixture of extracted analyte and solvent were vortexed and then poured into pre-weighed 60ml aluminum pans (Fisherbrand, Fisher Scientific). Pans were placed in a fume hood at ambient temperature for 24 hours to evaporate the solvent. Once completely dry, pans were reweighed and analyte concentration was calculated.

RESULTS

The yields of rubber by population are given in Table 1 and shown in Figure 2. The highest rubber yielding populations (red, Fig. 2) were MCUT (20.8 mg/g, Mill Creek, UT), in the Waco, TX area (14-18.2 mg/ g), and POCID (12 mg/g, Pocatello, ID). Several low yielding populations are shown in blue (Fig. 2): Redlands, OR, Preston, ID- Logan-UT, Capulin, NM - Eagle Nest, NM - Oslo, TX, Dodge City - Ellsworth, KS and Post - Quanah, TX.

It might be noted that the lowest elevation population (except San Diego) was Waco, TX and the highest elevation was MCUT (Salt Lake City, UT). Both of the areas were high in rubber concentration.

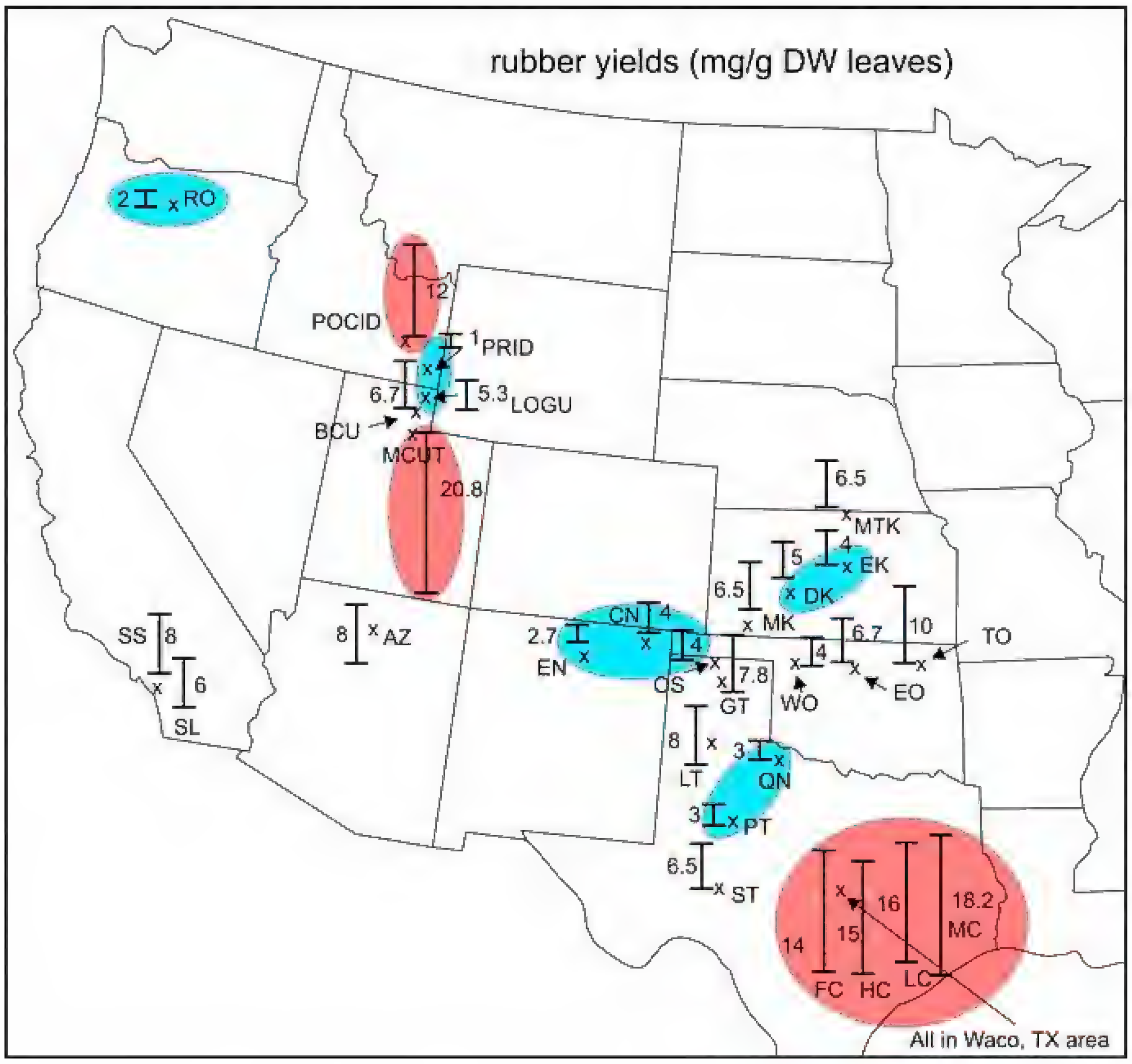

Figure 2. Geographic variation in yields of rubber (mg/ g DW leaves) from natural populations of *H. annuus*. Bar values are the population average. High yielding populations are in red, and low yielding are in blue.

The populations around Waco, TX (FC, HC, LC, MC) are rather uniform in their averages. The variation in northern UT and southern ID, is a mosiac with 2 low yielding populations (PRID, LOGU) flanked by 2 high yielding populations (MCUT, POCID).

To examine intra-populational variation, rubber yields for individual plants were mapped by population (Fig. 3). The highest yielding plant (42 mg/ g) was from the MCUT population (green, Fig. 3). Yields from plants in the MCUT population ranged from 10 to 42 mg/ g. The second highest yielding plant in this study (31 mg/ g) was from McLennan County, Texas. In the Waco, TX area (green, Fig. 3), yields ranged from 12 to 31 mg/ g. Large numbers of plants were analyzed from GT (Gruver, TX) and LT (Lake Tanglewood, TX), so frequency distribution graphs (yellow) were constructed. The GT population had a mode of 8 mg/ g and then tailed to 16 mg/ g (Fig. 3). The LT population appeared to be bi-modal with 3 plants with 2 mg/ g, 1 with 4 mg/ g, 1 with 6 mg/ g and then most with 8 - 14 mg/ g (Fig. 3).

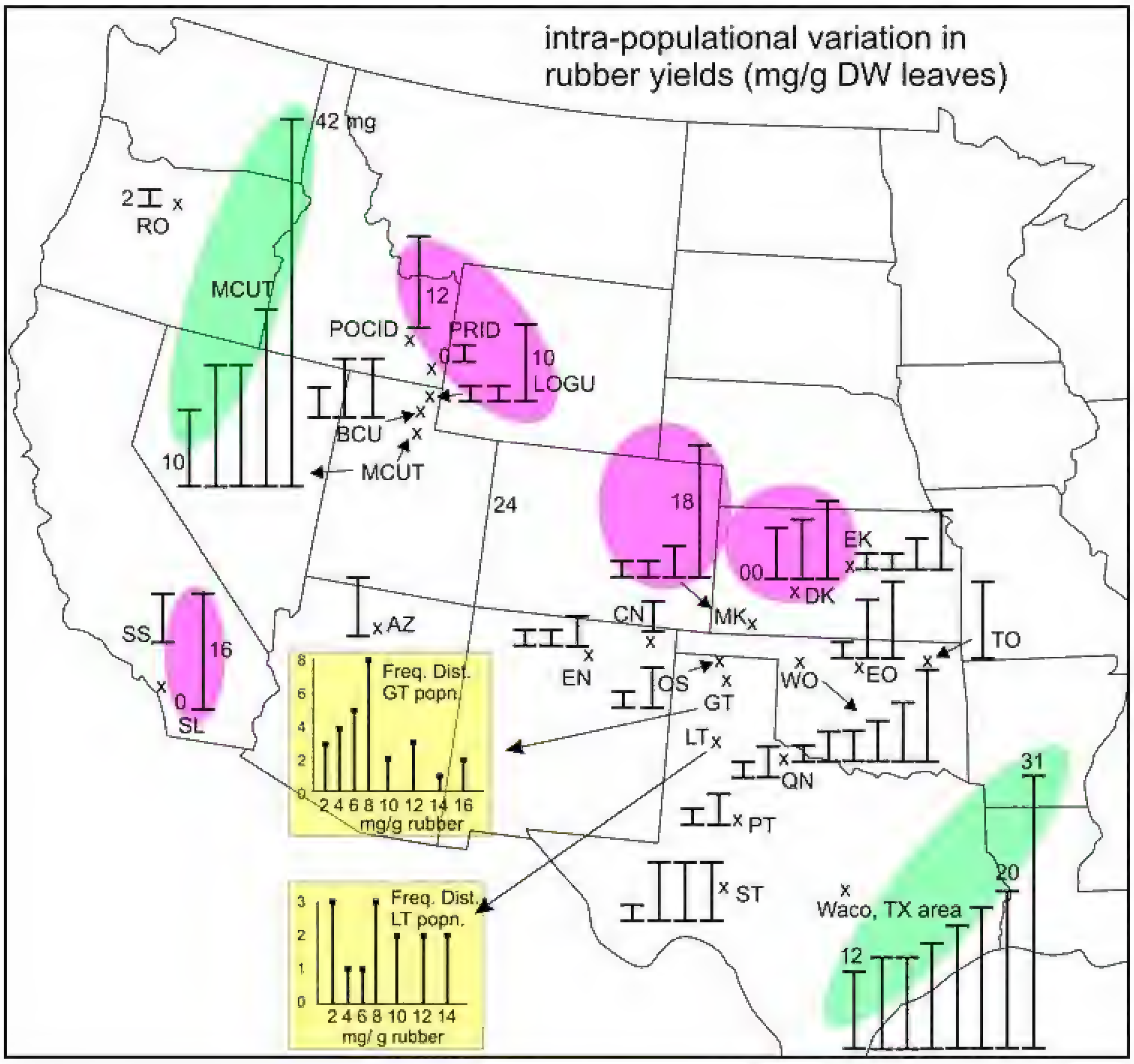

Figure 3. Intra-populational variation in rubber yields (mg/ g DW leaves). Note that populations GT and LT contained too many samples to show individually, so they as summarized in Frequency Distribution graphs (yellow). The high yielding individuals at MCUT (Mill Creek, UT) and in the Waco, TX area are shaded in green. Populations with polymorphic yields are shaded in magenta.

Several populations appeared to have a polymorphic (or chemotypes, low and high yields) production of rubber. In general, these populations (magenta, Fig. 3) contained individuals with no (zero detected) rubber, and plants with some or considerable amounts of rubber. For example, the DK (Dodge City, KS) population had 2 plants with 0 (none) and 3 plants with 7, 8, 10 mg/ g yields of rubber. The Meade, KS (MK) population had 2, 2, 4 mg/ g plants and one plant with 18 mg/g yield. Likewise, northern Utah - southern Idaho plants contained 0, 2, 2, 4, 10, 12 mg/ g which is suggestive of low- and high- rubber chemotypes. Additional research will be needed to fully ascertain if these chemotypes exist, or if the range of variation is continuous when additional samples are analyzed. Only three samples were analyzed from San Diego, CA (SL, SS), and they yielded 0, 6, and 18 mg/ g.

Plants that appear to have no rubber could provide useful parents when crossed with high yielding plants to examine the genetic basis of rubber yields in sunflowers (research in progress).

It is interesting to examine the distribution of HC yields (Fig. 1) vs. rubber yields (Fig. 2). Notice that the highest yielding HC populations (GT, LT, Fig. 1) are quite intermediate in their rubber yields (Fig. 2). Likewise, the high yielding rubber populations (FC, HC, LC, MC, Fig. 2) are just medium yielding HC populations (Fig. 1). However, the low HC yielding populations (AZ, EN, RO, PT, QN, WO, Fig. 1) are also low in rubber (Fig. 2). Because we have shown (Adams, et al. 2017b) that high yields of HC are induced by stress factors (insect, wind and disease conditions), it is not surprising that the high HC yielding populations do not necessarily correlate with high rubber yielding populations. In fact, the correlation between HC yields and rubber yields was found to be r= 0.195. Thus, only 3.8% of the variance in HC is explained by variation in rubber yields. Although the hydrocarbon fraction (HC) in *H. annuus* contain considerable amounts of terpenoids (Adams et al. 2017c; Gershenzon et al. 1981), the induction of mono-, sesqui-, di- and tri-terpenoids by stresses does not seem correlated the amount of rubber (a terpenoid polymer).

In summary, considerable geographic variation was found in the yields of rubber in native sunflowers (*H. annuus*) that varied from 0 (zero) to 42 mg/ g. At present (until plants are grown in a garden/ greenhouse), the role of genetics vs. environment is not known in sunflower rubber production. Nor is the range of geographic variation fully known for *H. annuus*. New collections in the western portion of the range of *H. annuus* are being analyzed and this will more fully complete the knowledge of geographic variation in the yields of rubber in natural populations.

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Table 1. Yields of ASE natural rubber (mg/g leaves DW) from natural sunflower (*H. annuus*) populations. Yields are rounded to nearest mg. Specimen collection numbers are of *Robert P. Adams* collection series.

popn id		ASE rubber Avg.	ASE rubber, individuals
AZ	15021 Camp Verde, AZ (10), 8/27/16 Thornburg	8.06	8
BCU	15022, Brigham City, UT, (10), 9/2/16	6.67	4,8,8
CN	14981 Capulin, NM (10), TeBeest ns	4	4
DK	14940 H. ann. (10), 7/7/16, Dodge City, KS	5.0	0,0,7,8,10
EK	14941 H. ann. (10), 7/7/16, Ellsworth, KS	4.0	2,2,4,8
EN	14980 Eagle Nest, NM,(10), TeBeest ns	2.67	2,2,4
EO	14943 H. ann. (10), 7/9/16, Enid, OK EOK (10)	6.67	2,8,10
FC	14977 Falls Co. Satin, (10), Holmes 16656	14	14
GT1	14952 H. ann., Gruver, TX, GT1 1-10, mi south 7/16/19	6.9	2,4,4,4,8,10,16
GT2	GT2 11-20, 1mi south 7/29/16	8.6	2,6,6,8,8,14,16
GT3	GT 21-30, 2 mi E, Rodeo Grounds 8/1/16	9.7	8,8,8,8,12,12,12
GT4	GT 31-40, 1 mi south 8/15/16	6.0	2,4,6,6,6,8,10
HC	14979 Hill Co., (10), Holmes 16661	15.0	12,18
LC	14978 Limestone Co. Mt. Calm, (10), Holmes 16658	16	16
LOGU	15023, Logan, UT, (10), 9/2/16	5.3	2,4,10
LT1	14947 H. annuus, Lake Tanglewood, TX LT (10), 7/12/16	7.7	2,6,8,8,8,10,12
LT2	Lake Tanglewood, LT2, (10), 2nd collection 7-20-16	8.3	2,2,4,10,12,14,14
MC	14976 McLennan Co. (10), Holmes 16654 1-10	18.2	10,12,18,20,31
MCUT	15026, Mill Creek, Salt Lake City, UT (10), 9/4/2016	20.8	10,14,14,24,42
MK	14939 H. annuus (10), 7/7/16,Meade, KS,	6.5	2,2,4,18
OS	14946 H. annuus (10), 7/12/16, Olso, TX native in grass	4	2,6
POCID	15025, Pocatello, ID, (10), 9/2/16	12	12
PRID	15024, Preston, UT, (10), 9/2/16	1.0	0,2
PSTX	14935 H. ann. (10), 6/4/16, Post, TX PST (10)	3	0,2,4
QN	14936 H. ann. (10), 6/5/16, Quanah, TX 6/5/16 QT (10)	3.0	2,4
ROR	15027, Redmond, OR, (10), 8/30/16	2	2
SL	14951 H. ann. San Diego,CA large lvs, (10), 7/6/16, Bartel SD8-1...10	8	0,16
SS	14950 H. ann. San Diego,CA small lvs, (10), 7/6/16, Bartel SD6-1...10	6	6
ST	14945 H. ann. (10), 7/12/16, (11) ex Sonora, TX (cult Oslo, TX) ex PI413168	6.5	2,8,8,8
TO	14942 H. ann. (10), 7/9/16, Tulsa, OK	10	10
WO	14944 H. ann. (10), 7/9/16, Woodward, OK smooth lvs, hybrid with cultivated sunflowers.	4.3	2,2,4,4,6,8

Appendix I Population locations.

Helianthus annuus L. below:

common along railroad and roadside in sandy soil. flowering. 5.3 mi SE of Post TX on US 84, 33° 01' 53" N, 101° 11' 25" W, 2300 ft, Date: 4 June 2016 County: Garza; State: TX
Coll. Robert P. Adams No. 14935

common along fence row and roadside in sandy soil. flowering.7 mi SE of Quanah, TX on US 287,34° 15' 57" N, 99° 36' 46" W, 1450 ft, Date: 5 June 2016 County: Hardeman; State: TX
Coll. Robert P. Adams No. 14936

1.5 mi s of Meade, on KS23, low area in edge of wheat field, 100s of plants in population, but generally uncommon. ~5% flowering. 37° 15' 49" N, 100° 20' 40" W, 2433 ft, Date: 7 July 2016; County: Meade; State: KS
Coll. Robert P. Adams No. 14939

8.5 mi NE of Dodge City, US 50, several on dirt piles of highway dept., but generally uncommon. ~5% flowering, 37° 47' 06" N, 99° 53' 14" W. 2534 ft. Date: 7 July 2016, County: Ford; State: KS
Coll. Robert P. Adams No. 14940

1.6 mi e of Ellsworth on KS140, on fence row on s side of wheat field, 20 plants, but generally uncommon. ~10% flowering. 38° 44' 24" N, 98° 11' 53" W, 1600 ft, Date: 7 July 2016, County: Ellsworth; State: KS
Coll. Robert P. Adams No. 14941

15 plants on disturbed area next to South Ash St. (just south of OK364), but generally uncommon, Jenks, OK (sw suburb of Tulsa). ~5% flowering. 36° 00' 57.85" N, 95° 58' 07.61" W, 613 ft, Date: 9 July 2016, County: Tulsa; State: OK
Coll. Robert P. Adams No. 14942

5.5 mi e of Enid on OK412,on fence row, side of wheat field, few plants but generally uncommon. ca 5% flowering, ~5% flowering. 36° 23' 51" N, 97° 46' 51" W, 1160 ft., Date: 9 July 2016, County: Garfield; State: OK
Coll. Robert P. Adams No. 14943

smooth leaves! 2.8 mi e of Woodward on OK412,on fence row, side of grass field, few plants but generally uncommon. ca 5% flowering mostly pre-flowering. 36° 25' 53" N, 99° 20' 28" W, 1880 ft., Date: 9 July 2016, County: Woodward; State: OK
Coll. Robert P. Adams No. 14944

cultivated at Oslo, TX, from seed (USDA PI413168-NC7) ex Sonora, TX. 80% flowering, 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft, Date: 12 July 2016, County: cult in Hansford; State: TX.
Coll. Robert P. Adams No. 14945

native in grassland, JP & Amy TeBeest farm, 1 mi. s of Oslo Lutheran Church. ~5% flowering. 36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft., Date: 12 July 2016, County: Hansford; State: TX
Coll. Robert P. Adams No. 14946

2- 3 ft plants, lots of resin on petioles and leaf veins, many sugar (black) ants, most with wilted leaves, very dry in July, common in native grass and on disturbed roadside, brush dump area, Lake Tanglewood, ~50% flowering, 35° 04' 23.7" N, 101° 47' 29.0" W, 3239 ft., Date: 12 July 2016, County: Randall; State: TX
Coll. Robert P. Adams No. 14947

cultivated at Oslo, TX, from seed (USDA PI 531028) ex Idaho, 80% flowering.36° 25' 12.3" N, 101° 31' 54.6" W, 3239 ft, Date: 12 July 2016, County: cult in Hansford; State: TX
Coll. Robert P. Adams No. 14948

plants 2' tall, with small leaves, along San Pasqual Rd, 33° 05' 08.2" N, 117° 01' 46.2" W, 353 ft.
Date: 6 July 2016, County: San Diego; State: CA, Coll. Jim A. Bartel 1636
Lab Acc. Robert P. Adams No. 14950

plants to 8' tall, with large leaves, along San Pasqual Rd, 33° 05' 08.2" N, 117° 01' 46.2" W, 353 ft/ Date: 8 July 2016, County: San Diego; State: CA, Coll. Jim. Bartel 1636
Lab Acc. Robert P. Adams No. 14951

2-3' tall, 10% flowering, lots of damage to leaves by grasshoppers, etc., some with many black (sugar) ants, copious resin at base of leaves, along fence row, on TX 206, 1-5:1.2 mi s, 6-10: 1.3 mi. s of Gruver, TX. 36° 14' 52" N, 101° 24' 52" W, 3161 ft, Date: 16 July 2016, County: Hansford; State: TX
Coll. Robert P. Adams No. 14952

cultivated, irrigated near Oslo, TX, on Slough farm. at R-5.1 stage. 36° 22' 42.17" N, 101° 37' 21.4" W, 3350 ft., leaves mostly smooth. Date: 17 July 2016, County: cult in Hansford; State: TX
Coll. Robert P. Adams No. 14953

Coll. Walter Holmes
(WCH16654) McLennan Co. 12th Street at Flat Creek, Robinson (Waco), 27 July 2016 , Walter Holmes
Lab Acc. Robert P. Adams 14976
(WCH 16656) Falls Co. near Satin on FR 434, prairie roadside, 28 July 2016, Walter Holmes
Lab Acc. Robert P. Adams 14977
(WCH 16658) Limestone Co. near jct of Limestone Co roads 102 and 106, south of Mt. Calm, prairie, 29 July 2016, Walter Holmes
Lab Acc. Robert P. Adams 14978
(WCH 16661) Hill Co. US Hwy 84, West of Mt. Calm near jct with West Somers Lane, 29 July 2016, Walter Holmes
Lab Acc. Robert P. Adams 14979

roadside waste area, Eagle Nest, NM, 36° 33.650' N, 105° 15.969' W, 8260 ft, Date: 8 Aug 2016, County: Colfax; State: New Mexico, Coll. Amy TeBeest
Lab acc. Robert P. Adams 14980

roadside waste area, Capulin (city), NM, some grasshopper damage, 36° 44.527' N, 104° 00.178' W, 6820 ft,
Date: 8 Aug 2016, County: Union: State: New Mexico, Coll. Amy TeBeest
Lab acc. Robert P. Adams 14981

cultivated at Oslo, TX, from seed (USDA PI1413033), ex Montrose, KS. Date: 2 Aug 2016, Coll. Amy TeBeest,
Lab acc. Robert P. Adams 14982

along roadsides. 16-18 mi east of Camp Verde on AZ 260. 34.489° N, 111.597° W, 5900 ft, Date: Aug. 27, 2016, County: Yavapai; State: AZ, Coll. David Thornburg ns,
Lab. acc. Robert P. Adams No. 15021
vacant lot behind new WalMart on disturbed soil. flowering and seeding, multiple branches. W1500S, 775W, Brigham City, UT, 41° 28' 57" N, 112° 01' 40" W, 4250 ft, Date: Sept. 2, 2016, County: Boxelder; State: UT
Coll. Robert P. Adams No. 15022

vacant lot behind new stores on disturbed soil on US 91 and E2000N, flowering and seeding, multiple branches. Logan, UT, 41° 46' 09" N, 111° 49' 59" W, 4506 ft, Date: Sept. 2, 2016, County: Cache; State: Utah
Coll. Robert P. Adams No. 15023

vacant lot in new subdivision on disturbed soil off of OR hwy 34/36 & just on n edge of Preston, flowering and seeding, multiple branches. 42° 06' 40" N, 111° 52' 01" W, 4703 ft, Date: Sept. 2, 2016, County: Franklin; State: Idaho
Coll. Robert P. Adams No. 15024

next to sidewalk, on slope, next to freeway (I15) access south, flowering and seeding, multiple branches, Pocatello, ID. 42° 52' 49" N, 112°' 25' 35" W, 4625 ft, Date: Sept. 2, 2016, County: Bannock; State: Idaho
Coll. Robert P. Adams No. 15025

next to sidewalk, flowering and seeding, multiple branches. common along sidewalks, Mill Creek, UT. s side of I80 on 2000 E, east side of 2000E. 42° 52' 49" N, 112°' 25' 35" W, 4625 ft, Date: Sept. 3, 2016, County: Salt Lake; State: Utah
Coll. Robert P. Adams No. 15026

disturbed area, vacant on SW Airport Way, ~373m sse of jct SW Airport Way & Veterans Way. Redmond, OR, 44° 15' 30" N, 121°' 09' 54" W, 3035 ft, Date: Sept. 3, 2016, County: Redmond; State: Oregon
Coll. Mark R. Corbet, ns, Lab Acc. Robert P. Adams No. 15027

disturbed area, Neil Rd and west frontage road on I580, s of Reno, NV. 39° 28' 11.6" N, 119°' 47' 20.4" W, 4485 ft, Date: Sept. 5, 2016, County: Washoe; State: Nevada
Coll. Chauncey Parker, ns, Lab Acc. Robert P. Adams No. 15029.

Arceuthobium microcarpum* (Viscaceae): morphological evidence for continued species recognition and discrimination from *Arceuthobium campylopodum

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ABSTRACT

Western spruce dwarf mistletoe (*Arceuthobium microcarpum*, Viscaceae) parasitizes Engelmann spruce (*Picea engelmannii*) and blue spruce (*P. pungens*) in Arizona and New Mexico. In Arizona, a subspecies (*A. microcarpum* subsp. *aristatae*) also parasitizes Rocky Mountain bristlecone pine (*Pinus aristata*). Although *A. microcarpum* was first segregated from western dwarf mistletoe (*A. campylopodum*) by Engelmann in 1878, its taxonomic classification has undergone several recombinations; the most recent making it a subspecies of *A. campylopodum.* Because the morphologies of *A. campylopodum* and the subspecies of *A. microcarpum* have not been compared using multivariate statistical analyses, we undertook this study. We used morphological data available from our previous taxonomic investigations of these taxa as well as additional data collected for *A. microcarpum* in 2017. Statistical comparisons provided herein demonstrated that *A. microcarpum* can be reliably segregated from *A. campylopodum* using plant heights, plant basal diameters, flower diameters, and fruit and seed dimensions. We were also able to distinguish between the subspecies of *A. microcarpum*, but as expected the differences were not as great as those between *A. campylopodum* and both subspecies of *A. microcarpum.* Furthermore, the host affinities of these taxa clearly distinguished them from each other. Therefore, we recommend that *A. microcarpum* continue to be recognized as a distinct species from *A. campylopodum* and that the subspecies currently recognized under *A. microcarpum* be maintained. Morphological differences between these dwarf mistletoes were summarized. Published on-line www.phytologia.org *Phytologia 100(1): 71-90 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: dwarf mistletoes, morphological characters, multivariate analyses, *Picea*, *Pinus.*

Western spruce dwarf mistletoe (*Arceuthobium microcarpum* (Engelmann) Hawksworth & Wiens (Viscaceae) is a parasite of Engelmann spruce (*Picea engelmannii* Parry ex Engelmann) and blue spruce (*P. pungens* Engelmann) in Arizona and New Mexico (Mathiasen and Hawksworth 1980, Hawksworth and Wiens 1996). Although it is only locally abundant in the White Mountains, Arizona, it is associated with increased mortality of both of its principal hosts there (Mathiasen et al. 1986). Populations of *A. microcarpum* that severely infect Rocky Mountain bristlecone pine (*Pinus aristata* Engelmann) on the San Francisco Peaks near Flagstaff, Arizona have been classified as *A. microcarpum* (Engelmann) Hawksworth & Wiens subsp. *aristatae* Scott & Mathiasen based on morphological and host range differences with other populations of *A. microcarpum* (Scott and Mathiasen 2009).

Arceuthobium microcarpum was initially segregated from *A. campylopodum* by Engelmann in 1878 as a variant of *A. douglasii* Engelmann based on a collection by Gilbert from Engelmann spruce near Sierra Blanca in the White Mountains, Arizona (Apache County). In 1915, it was reclassified as *Razoumofskya microcarpa* (Engelmann) Wooton & Standley (Hawksworth and Wiens 1996). In the first monograph of *Arceuthobium* in the United States, Gill (1935) classified it as the host-form of *A. campylopodum* that parasitized spruces (*Picea* spp.): *A. campylopodum* Engelmann forma *microcarpum* (Engelmann) Gill. This classification was used until Hawksworth and Wiens (1970) recombined Gill's forma *microcarpum* as *A. microcarpum.* Hawksworth and Wiens (1972, 1996) maintained the classification of *A. microcarpum* as a species and this treatment has been followed in most, if not all, studies of this dwarf mistletoe since 1972 (e.g. Acciavatti and Weiss 1974; Crawford and Hawksworth 1979; Hawksworth and Mathiasen 1980; Martin and Hutchins 1980; Mathiasen et al. 1986; Lynch 2004; Scott and Mathiasen 2009).

The taxonomic classification of *Arceuthobium microcarpum* recently became a topic of debate primarily because of molecular data (Nickrent et al. 2004). The molecular markers examined thus far indicated *A. microcarpum* is closely related to *A. campylopodum*, and therefore, its segregation from *A. campylopodum* as a distinct species has been questioned (Nickrent 1996; Nickrent et al. 2004). Based on molecular data and the morphological similarities between species in section *Campylopoda* Hawksworth & Wiens, series *Campylopoda*, Nickrent (2012, 2016) recombined *A. microcarpum* as a subspecies of *A. campylopodum.* Because of these recent treatments of *A. microcarpum* as a subsp. of *A. campylopodum* and because the morphologies of *A. campylopodum* and the subspecies of *A. microcarpum* have not been directly compared, we undertook this study using morphological data we had available from previous studies of these taxa (Mathiasen 1977; Mathiasen and Hawksworth 1980; Scott and Mathiasen 2009; Mathiasen and Kenaley 2015). In addition, we collected additional morphological data for *A. microcarpum* subsp. *microcarpum* in 2017. Our objective was to compare the morphologies of these taxa to determine if the classification of the subspecies of *A. microcarpum* or the classification of *A. microcarpum* as a subspecies of *A. campylopodum* were supported by more robust multivariate statistical analyses than have been used in previous studies by Mathiasen and Hawksworth (1980) and Scott and Mathiasen (2009).

MATERIALS AND METHODS

We collected morphological data for *Arceuthobium campylopodum* from 60 populations (30 each from *Pinus ponderosa* Douglas ex Lawson & C. Lawson and *P. jeffreyi* Greville & Balfour) from throughout most of its geographic range (Mathiasen and Kenaley 2015) (Figure 1). Mathiasen and Hawksworth (1980) sampled 26 populations of *A. microcarpum* subsp. *microcarpum* from throughout its geographic distribution in Arizona and from the Mogollon Mountains, New Mexico from 1975-1976 (and see Appendix A in Mathiasen 1977). Additional morphological data for *A. microcarpum* were collected in 2006-2007 from 12 populations in Arizona by Scott and Mathiasen (2009); six of those populations were from the White Mountains, Arizona, two were from the North Rim Grand Canyon, and four were from either the San Francisco Peaks or nearby Kendrick Peak, Arizona (Figure 2). In 2017, additional morphological data were collected for *A. microcarpum* subsp. *microcarpum* from the White Mountains (6 populations), the Pinaleno Mountains (two populations), the Mogollon Mountains (one population), and the North Rim Grand Canyon (one population) (Figure 2). Five of the 10 populations sampled in 2017 were the same as those sampled in 2006-2007. Because the 2011 Wallow Fire destroyed several *A. microcarpum* subsp. *microcarpum* populations in the White Mountains, not all of the same populations sampled in 2006-2007 could be sampled in 2017. Large wildfires have also burned in the Mogollon and Pinaleno Mountains in recent years; hence, populations of subsp. *microcarpum* were only sampled in a few locations in those areas. Furthermore, we only used the morphological data collected by Scott and Mathiasen (2009) for subsp. *aristatae* because the 2010 Schultz Fire drastically reduced the extent of the subsp. *aristatae* population on Schultz Peak, therefore, no additional morphological measurements were completed for this subspecies. Voucher specimens for *A. campylopodum* (Mathiasen and Kenaley 2015) and *A. microcarpum* (Scott and Mathiasen 2009) consisting of the mistletoe with host material were deposited at the Deaver Herbarium, Northern Arizona University, Flagstaff (ASC), or the University of Arizona Herbarium, Tucson (ARIZ). Voucher and specific population data, including GPS coordinates, for the 2009 and 2015 studies have been archived electronically in SEINet (Southwest Environmental Information Network 2017: http://swbiodiversity.org/portal/index.php). Voucher specimens for the subsp. *microcarpum* populations sampled in 2017 were deposited at the Rancho Santa Ana Botanical Gardens (RSA).

In 1975 and 2006-2007, the following morphological characters for male plants were measured for the subspecies of *Arceuthobium microcarpum*: dominant plant height, dominant plant basal diameter, flower diameter, anther diameter, petal lobe length and width, and distance from the outer edge of the anther to the tip of the petal lobe. The following morphological characters were measured for female plants: dominant plant height, dominant plant basal diameter, length and width of both fruits and seeds. The color of plants, fruits, and seeds were also recorded. Measurements in 1975 were completed with a stereoscope microscope fitted with a micrometer and all other morphological characters were measured using a 10x hand lens with a micrometer (Bausch & Lomb, Bridgewater, NJ) to the nearest 0.1 mm. Male plants were collected during the peak of anthesis and female plants were collected when fruits were mature. Over 20 male or female plants were collected for each population and morphological measurements were completed using ten randomly selected plants for each population sampled. For some populations, more than 10 staminate flowers or fruits/seeds were measured (20). Sample sizes for most morphological characters measured varied between the two species sampled because of the number of populations sampled and the number of plants, staminate flowers, and fruits/seeds measured per population also varied.

For each population of *Arceuthobium campylopodum*, 10–20 male and 10–20 female infections (infected branches) were collected separately and the dominant plant (largest plant) from each infection was used for morphological measurements. The following morphological characters were measured: 1) height, basal diameter, third internode length and width, and color of male and female plants; 2) mature fruit length, width, and color; 3) seed length, width and color; 4) length and width of staminate spikes; 5) staminate flower diameters for 3- and 4-merous flowers; 6) length and width of staminate flower petals; and 7) anther diameter and anther distance from the petal tip. Plant heights were measured to the nearest 0.1 cm and all other measurements were made to the nearest 0.1 mm. Plants were usually measured within 12-h, but no later than 24-h after collection. Only plants that were still attached to their host's branch and fully turgid were measured. Measurements were made using a digital caliper (Mitutoyo America Corp., Aurora, IL) and a 7X hand lens equipped with a micrometer (Bausch & Lomb, Bridgewater, NJ). The basal diameter of plants was measured at the point where the plant was attached to the host branch. Staminate spike and flower measurements were made during the peak of anthesis and fruit and seed measurements were made during the peak of seed dispersal.

Statistical Analyses

One-way analysis of variance (1-way ANOVA) was performed to examine the variance in each of the male and female morphological characters between *Arceuthobium microcarpum* and *A. campylopodum* ($\alpha = 0.05$). Mean differences among morphologic characters of female and male plants across taxa were assessed using a post-hoc Tukey's honestly significant difference (HSD; $\alpha = 0.05$) test. Dunnett's tests were also executed separately to determine whether means for each female and male morphologic character were significantly different when comparing each of the subspecies of *A. microcarpum* individually to *A. campylopodum*.

Morphological differences across six female and seven male characters among taxa – *Arceuthobium campylopodum*, *A. microcarpum* subsp. *aristatae*, and *A. microcarpum* subsp. *microcarpum* – were tested simultaneously and separately by sex using multivariate analysis of variance

(MANOVA). Standard and forward-stepwise quadratic discriminant function analyses (DFA) were also performed to determine whether female and male plants could be segregated by taxonomic affiliation (i.e., field diagnosis vs. predicted taxonomic membership) utilizing either female or male plant morphology (Quinn and Keough 2002). Because previous molecular phylogenetic analyses suggested that *A. microcarpum* and *A. campylopodum* may be conspecific (i.e., the same species differing only by principal host; Nickrent et al. 2004), DFAs for female and male plants were performed separately using complete morphological records for each taxon and setting the prior probability to 0.3333 per taxon rather than according to field diagnosis and, hence, sample size. Standardized correlation coefficients (SCC) for female and male morphologies were calculated as part of the standard DFA to determine the overall contribution of each morphologic character to the discriminant function; thereby, providing the principal female or male character(s) separating the dwarf mistletoes. Thereafter, the standard DFAs were validated by resampling separately the original (complete) data set for female and male plants; selecting at random 50 complete records per taxon and re-executing the DFA using a full-model (i.e., 6 female or 7 male characters simultaneously). Forward-stepwise DFA was executed separately for female and male plants to assess the combinations of female and male morphologies resulting in the highest precision (%, predicted/field determined) in taxonomic membership, and hence, maximize differences among taxa. Only the diameter of 3-merous male flowers was included in the DFAs because few 4-merous flowers were measured for *A. microcarpum*. One-way and multivariate analyses of variances, multiple comparisons of mean differences, and DFAs were computed in JMP Pro v13.0 (SAS Institute, Cary, North Carolina, USA). Ninety-five percent (95%) confidence intervals ($\alpha = 0.05$) were also calculated in lieu of standard deviations and errors.

RESULTS

The means for every morphological character measured were significantly larger for *Arceuthobium campylopodum* than for either of the subspecies of *A. microcarpum*; this was demonstrated by ANOVA and corresponding Tukey's HSD and Dunnett's tests (Table 1). The principal characters found to differentiate between *A. campylopodum* and *A. microcarpum* and its subspecies *aristatae* are summarized in Table 2. Another striking dissimilarity between *A. campylopodum* and the subspecies of *A. microcarpum* was that the latter taxa often formed 2-merous staminate flowers; this was observed many times while measuring male flowers in 1975, 2007, and 2017. In addition, staminate flowers with five petals were also observed for *A. microcarpum*, although rarely and, interestingly, one male plant was found that produced only 5-merous flowers with ten mature, open flowers. Unlike *A. microcarpum*, staminate flowers of *A. campylopodum* with only two or five petals were never observed; it consistently formed only 3- and 4-merous flowers in approximately equal proportions.

In contrast to the large morphological differences between *Arceuthobium campylopodum* and the subspecies of *A. microcarpum*, the subspecies were morphologically similar (Table 1). The mean heights of both male and female plants were significantly different as were the mean basal diameters. Mean diameters of 3- and 4-merous flowers were not significantly different, but sample sizes for 4-merous staminate flowers were relatively small. The mean length and width of petals, anther diameters, and the distance to the tip of petals were also not significantly different for the subspecies. However, mean fruit length and width were significantly different as was seed width; mean seed length was not significantly different between the subspecies.

Multivariate analysis of variance (MANOVA) of complete records for female and male plants of *Arceuthobium campylopodum*, *A. microcarpum* subsp. *aristatae,* and *A. microcarpum* subsp. *microcarpum* revealed significant differences in the overall morphology among taxa for female (Wilks' $\lambda = 0.0928$, Approximant $F_{12,\,1786} = 339.7$, $P < 0.0001$; Pillai's Trace= 1.05, Approximant $F_{12,\,1788} = 166.2$, $P < 0.0001$) and male plants (Wilks' $\lambda = 0.1975$, Approximant $F_{14,\,1488} = 132.9$, $P < 0.0001$; Pillai's Trace= 0.9373, Approximant $F_{14,\,2020} = 93.9$, $P < 0.0001$). Standard DFA utilizing six female and seven male morphological characters (i.e., full-models) resulted in the correct classification (%, predicted/field diagnosed) of 96.1% and 89.1% of female and male plants, respectively (Table 3; Figure 3). Female plants of *A. campylopodum* determined a priori via field diagnosis were assigned correctly to *A. campylopodum* 100% of the time, whereas ≤ 1% of female *A. microcarpum* subsp. *aristatae* (0.9%) and subsp. *microcarpum* (1.0%) were classified to *A. campylopodum* when considering complete morphologic data (Table 4). Moreover, the taxonomic membership for female plants of *A. microcarpum* subsp. *aristatae* (96.3%) and *microcarpum* (90.1%) was predicted correctly ≥ 90.1% of the time. The first and second canonical (c1 and c2) explained 97.6% and 2.4% of the variation among female plants, respectively (Table 5; Figure 3); wherein, fruit length (SCC$_{c1, c2}$= 0.62, -0.05), plant height (SCC$_{c1, c2}$= 0.20, -1.0), seed length (SCC$_{c1, c2}$= 0.45, 0.01), and basal diameter (SCC$_{c1, c2}$= 0.30, 0.27) contributed most to the separation of *A. campylopodum* and the subspecies of *A. microcarpum*. Fruit (SCC$_{c1, c2}$= 0.11, 0.20) and seed width (SCC$_{c1, c2}$= 0.06, 0.34) contributed least to the discrimination of taxa. Standard DFA of female plant morphology was also supported by the stepwise DFA of female plant parts (Table 3), indicating that ≥ 88.2% of *A. campylopodum* (100%), *A. microcarpum* subsp. *aristatae* (98.1%), and *A. microcarpum* subsp. *microcarpum* (88.2%) were classified correctly to taxonomic membership when considering only fruit length, plant height, seed length, and basal diameter. Further examination of the female stepwise DFA results also revealed that *A. campylopodum* (99.2%) and the subspecies of *A. microcarpum* (98.1%, 84.7%) could be effectively delineated morphologically using only two of the four aforementioned female characters – fruit length and plant height. Resampling the female dataset (50 randomly-selected, complete records per taxon) and executing a full-model DFA also yielded nearly identical results (Table 5; Figure 3); classifying correctly 98.0% (147/150) of female plants and readily delimiting *A. campylopodum* from *A. microcarpum* sensu lato. Likewise, as with utilizing complete records, the female DFA with resampled data segregated clearly *A. microcarpum* subsp. *aristatae* and subsp. *microcarpum* (Figure 3). Means and associated 95% confidence intervals for morphological characters of female plants by predicted species according to full-model DFA are presented in Table 6.

Determining taxonomic membership via DFA across seven male plant characters was less precise when compared to the DFA utilizing female morphologies; resulting in 89.1% of all complete male records being assigned correctly to their taxonomic identity (Table 3). However, the first and second canonical discriminant functions for the full-model DFA of male plant morphology explained 93.6% and 6.4%, respectively, of the variation among *Arceuthobium campylopodum*, *A. microcarpum* subsp. *aristatae*, and *A. microcarpum* subsp. *microcarpum*. The seven percent (7%) reduction in the total correct classification between female and male standard DFAs was in large part due to the misclassification of male *A. microcarpum* to their subspecific membership (Table 4). Field-determined male plants of *A. campylopodum* were consistently and correctly classified to *A. campylopodum* (97.3%), whereas, 10.6% of male *A. microcarpum* subsp. *microcarpum* were incorrectly assigned to subsp. *aristatae* and, conversely, 20.6% of male *A. microcarpum* subsp. *aristatae* were predicted to subsp. *microcarpum* rather than subsp. *aristatae* (Table 4). The two subspecies of *A. microcarpum*, however, were only predicted to *A. campylopodum* ≤ 2.7% of the time and, thus, male plants of *A. microcarpum* sensu lato were readily segregated morphologically from male *A. campylopodum*. Based on standardized correlation coefficients (SCC) for the first and second canonicals (c1 and c2), male characters contributing most to predicting taxonomic membership were basal diameter (SCC$_{c1, c2}$= 0.72, 0.63), diameter of 3-merous flowers (SCC$_{c1, c2}$= 0.63, 0.08), and plant height (SCC$_{c1, c2}$= 0.03, -1.2). In fact, limiting the male DFA model to only basal diameter, 3-merous flower diameter, and plant height resulted in the correct classification of 95.0% and 89.4% of male *A. campylopodum* and *A. microcarpum* subsp. *aristatae*, respectively (Table 3). However, executing a full-model DFA – incorporating petal length (SCC$_{c1, c2}$= -0.15, 0.17), anther distance to tip (SCC$_{c1, c2}$= 0.06, 0.01), and anther diameter (SCC$_{c1, c2}$= 0.05, -0.01) – was necessary to maximize morphological differences among taxa, particularly between subspecies of *A. microcarpum* (Table 3). Discriminant function analysis using a random sample of male records (resampled dataset) successfully validated the full-model DFA with complete records: effectively separating *A. campylopodum* and *A. microcarpum* sensu lato, yet, providing limited support to the separation of *A. microcarpum* subsp.

aristatae and subsp. *microcarpum* (Figure 3). Multivariate means and 95% confidence ellipses for male plants of the subspecies of *A. microcarpum* were discrete in ordination space for DFAs executed with either the complete or resampled dataset, however, as evident by the overlapping 50% contour ellipses, multiple specimens of male *A. microcarpum* subsp. *microcarpum* shared morphologies consistent with those predicted for subsp. *aristatae* (Table 6; Figure 3).

DISCUSSION

Classifying *Arceuthobium microcarpum* as the same species or as a subspecies of *A. campylopodum* is not supported by our analyses of the morphological characters we measured for these taxa. Both of these species can be reliably identified by differences in their plant heights, basal diameters, flower diameters, and fruit length and width (Table 2). All of the morphological characters measured were significantly larger for *A. campylopodum* than those of *A. microcarpum*; *A. microcarpum* and its subspecies *aristatae* form much smaller and thinner plants. Multivariate analysis of variance as well as standard and stepwise DFAs of female and male plant characters demonstrated that *A. campylopodum* and *A. microcarpum* are morphologically distinct and can be effectively predicted to taxonomic membership using as few as two female (fruit length and plant height) and three male characters (basal diameter, 3-merous flower diameter, and plant height).

Another morphological difference between *Arceuthobium campylopodum* and *A. microcarpum* is the number of petals formed on staminate flowers; *A. campylopodum* consistently forms 3- and 4-merous flowers in approximately equal frequencies, but *A. microcarpum* consistently forms predominantly 3-merous flowers and only occasionally forms 4-merous flowers. In addition, *A. microcarpum* will occasionally form 2-merous and rarely 5-merous flowers, whereas, 2- and 5-merous flowers have not been reported previously for *A. campylopodum* (Hawksworth and Wiens 1996; Mathiasen and Kenaley 2015).

Although the morphologies of the subspecies of *Arceuthobium microcarpum* were similar, there were a few significant univariate differences between them (Table 2) and, using multivariate approaches, female plants between the two subspecies were readily delimited to taxonomic membership (i.e., actual vs. predicted; Tables 3 and 4). However, male plants of subsp. *microcarpum* (20.6%) were often misclassified to subsp. *aristatae* (Table 4; Figure 3) and the predicted male morphologies between subspecies were nearly identical – differing only by basal diameter and plant height (Table 6). Collectively, female and male plants of subsp. *aristatae* are much smaller than those of subsp. *microcarpum*. In addition, the fruits are smaller for subsp. *aristatae* when compared to those of subsp. *microcarpum*.

Peak anthesis for subsp. *aristatae* occurred one to two weeks earlier on the San Francisco Peaks than for subsp. *microcarpum* in the White Mountains in 2006 and 2007 (Scott and Mathiasen 2009). Furthermore, seed dispersal of subsp. *aristatae* also starts and ends one to two weeks earlier on the San Francisco Peaks than seed dispersal of subsp. *microcarpum* in the White Mountains. Observations of phenology in 1975 also found that subsp. *aristatae* flowers and disperses seed earlier than subsp. *microcarpum* (Mathiasen & Hawksworth 1980).

The host range of *Arceuthobium microcarpum* subsp. *microcarpum* is quite distinct from *A. campylopodum*; its principal hosts are Engelmann and blue spruce and it rarely infects corkbark fir (*Abies lasiocarpa* (Hooker) Nuttall var. *arizonica* (Merriam) Lemmon). Rocky Mountain ponderosa pine (*Pinus ponderosa* Douglas ex Lawson & C. Lawson var. *scopulorum* Engelmann) is immune to infection by *A. microcarpum* (Hawksworth and Wiens 1996). The principal hosts of *A. campylopodum* are ponderosa and Jeffrey pines and it has never been reported to infect any species of spruce (Hawksworth and Wiens 1996). Even though *A. microcarpum* subsp. *aristatae* parasitizes bristlecone pine as its principal host and rarely parasitizes southwestern white pine (*P. strobiformis* Engelmann) (Mathiasen and Hawksworth 1980), these white pines are in subgenus *Strobus* Lemmon and are not closely related to ponderosa or Jeffrey pines which are hard pines in subgenus *Pinus* L. Therefore, the host affinities of *A. campylopodum* and *A. microcarpum* are distinct and support their classification as separate species. Quantitative data on the susceptibility of Engelmann spruce to *A. microcarpum* subsp. *aristatae* demonstrated that it is much less susceptible (an occasional host) than bristlecone pine (Mathiasen and Hawksworth 1980, Scott and Mathiasen 2009). This major difference in the susceptibility of Engelmann spruce between the subspecies of *A. microcarpum* was further support for the classification of the populations parasitizing bristlecone pine in northern Arizona at the subspecific level.

Although the flowering periods of *Arceuthobium microcarpum* and *A. campylopodum* overlap in August and early September (Mathiasen and Hawksworth 1980; Hawksworth and Wiens 1996; Scott and Mathiasen 2009), these taxa are geographically isolated by approximately 300 km. Hence, cross pollination and hybridization is precluded by their geographic isolation, but it is unknown how long populations of these mistletoes have been separated. However, it is unlikely their distributions have overlapped even in the Pleistocene because *A. microcarpum* is not found in the spruce-fir forests north of Arizona in Nevada, Utah, or Colorado. Although southwestern dwarf mistletoe (*A. vaginatum* (Wildenow) Presl subsp. *cryptopodum* (Engelmann) Hawksworth & Wiens, the common parasite of Rocky Mountain ponderosa pine in the Southwest, is frequently sympatric with *A. microcarpum*, *A. vaginatum* flowers in the spring (April-May), not in the late summer. Therefore, the dwarf mistletoes that are principal parasites of ponderosa pine in the United States (*A. campylopodum* and *A. vaginatum*) are either geographically isolated from *A. microcarpum* or prevented from crossing with it by differences in flowering periods.

Additional evidence that *Arceuthobium microcarpum* warrants separation from *A. campylopodum* at the species level was presented by Crawford and Hawksworth (1979). Their analyses of the flavonoid chemistry of *Arceuthobium* found that *A. microcarpum* had the most distinctive flavonoid profile consisting of six compounds; while *A. campylopodum* contained only two of the flavonoid compounds detected. Crawford and Hawksworth (1979), therefore, maintained the unique flavonoid profile of *A. microcarpum* strongly supported its classification as a separate species.

Considering the analyses of the morphological data we present here for *Arceuthobium microcarpum* and *A. campylopodum* and the major discontinuities in the host affinities of these taxa, we maintain that it is more consistent with other specific classifications of dwarf mistletoes to continue classifying these taxa as species (Hawksworth and Wiens 1996). Our morphological analyses has demonstrated that these species are readily separated using several characters and field observations of their host affinities also demonstrated that they are genetically distinct in that they parasitize taxonomically distinct members of the Pinaceae as their principal hosts (Table 2). Our analyses also supported the continued classification of the mistletoe populations on spruce in Arizona and New Mexico as a separate subspecies of *A. microcarpum* than the populations that parasitize bristlecone pine in northern Arizona (subsp. *aristatae*).

Classifying *Arceuthobium microcarpum* populations as a subspecies of *A. campylopodum* as proposed by Nickrent (2012, 2016) is clearly not supported by this study. Nickrent's rationale for his treatment was based on his molecular findings (Nickrent 1996, Nickrent et al. 2004) and his conclusion that the species recognized by Hawksworth and Wiens (1996) in ser. *Campylopoda* represented ecotypes of *A. campylopodum*, including *A. microcarpum*. Ecotypes are considered to be an experimental category and their adaptive characteristics have to be empirically proven (Davis and Heywood 1965). Moreover, in the broadest sense and in the absence of experimental testing, ecotypes historically have been defined as intraspecies populations that are interfertile and possess multiple adaptive morphological and/or physiological traits that are quantifiable in nature (Turrill 1946). With the advent of molecular and genomic tools over the last four decades, the latter definition of ecotype has been amended to include not only intraspecific populations distinguishable by adaptive phenotypic and/or physiological variation for sustained life and reproduction in a particular environment but also allele frequencies across polymorphic loci (Lowry 2012). However, Nickrent (2012) did not provide any experimental evidence – morphological, physiological, or genetic – that substantiated his contention that species in ser. *Campylopoda* should be considered ecotypes. He pointed to the fact that there was no definitive data available on the interfertility of the taxa in the series, but did not discuss that ecotypes are usually proven to be interfertile to some degree by experimental crossings (Clausen et al. 1940, 1948; Davis and Heywood 1965). In addition, Nickrent (2012) did not provide data supporting his supposition that seeds from female plants growing on susceptible hosts die when placed on non-susceptible hosts. Nickrent (2012) also speculated that there appeared to be a correlation with plant height of the taxa in ser. *Campylopoda* with elevation; larger plants occurred at lower elevations and smaller at high elevations. However, he failed to provide any experimental evidence that supported this assertion as well. Based on his definition of ecotypes in *Arceuthobium*, Nickrent (2012) recombined nearly all of the species in ser. *Campylopoda* as subspecies of *A. campylopodum* and he followed this classification in his treatment for *Arceuthobium* in the Flora of North America (Nickrent 2016). The application of Nickrent's (2012, 2016) treatment of *A. microcarpum* as a subspecies of *A. campylopodum* precludes the recognition of *A. microcarpum* subsp. *aristatae*. Evidently, Nickrent (2012) was not aware of the description of subsp. *aristatae* by Scott and Mathiasen (2009) because it was not listed under his nomenclatural summary for his recombination *A. campylopodum* subsp. *microcarpum* (Engelmann) Nickrent (Nickrent 2012, pg. 10). Therefore, Nickrent (2012) did not consider all of the available morphological and physiological data that had been published for *A. microcarpum* and only used Hawksworth and Wiens (1996) as the source of his data. Here, we have provided further evidence that *A. microcarpum* is morphologically distinct from *A. campylopodum*. Furthermore, our results do not support the Nickrent (2012, 2016) treatment which ignores the existence of subsp. *aristatae* parasitizing bristlecone pine in northern Arizona.

Without having described and named *Arceuthobium microcarpum* subsp. *aristatae*, this rare dwarf mistletoe cannot be protected. From the conservation biology perspective, it is prudent to name plants that can be demonstrated to be genetically, morphologically, and/or physiologically different, even if the differences are cryptic, than to group them as one species (Baldwin 2000). Grouping them together gives the sometimes incorrect impression that a species which actually consists of many genetically different, but morphologically similar populations, are widespread and abundant when in reality they consist of many different species or infraspecific taxa with very limited distributions that deserve recognition from a conservation biology/biodiversity perspective (Baldwin 2000, Simpson 2010). Following the classification of most of the taxa in ser. *Campylopoda* as subspecies of *A. campylopodum* as proposed by Nickrent (2012, 2016) prevents the recognition and possibly the conservation of many populations of *Arceuthobium* in the western United States with similar morphologies, but different host affinities, including subsp. *aristatae* (Mathiasen and Kenaley 2015, 2016, 2017).

The recent increases in both size and frequency of high-severity fires across the entire western United States has been especially evident across Arizona (Westerling et al. 2006; Westerling 2016), affecting a large area of the geographic distribution for both subspecies of *Arceuthobium microcarpum*. Due to the parasitic habit of dwarf mistletoes and the high probability for low survival of host trees in high-severity wildfires, these fires can greatly reduce dwarf mistletoe populations (Alexander and Hawksworth 1975; Harrington and Hawksworth 1990; Shaw and Agne 2017). Therefore, the distribution of *A. microcarpum* subsp. *aristatae* needs to be evaluated to determine the impacts that several wildfires have had on its populations on Kendrick and Schultz Peaks. In 2000, the Pumpkin Fire burned throughout much of the Kendrick Peak Wilderness Area as did the Boundary Fire in 2017. In 2010, the Schultz Fire burned in a mosaic across Schultz Peak. According to Monitoring Trends in Burn Severity wildfire data (Eidenshink et al. 2007) for the Shultz Fire, approximately one-third of the entire *Arceuthobium microcarpum* subsp. a*ristatae* population burned as high-severity, thus killing many of the dwarf mistletoe-infected bristlecone pines on Schultz Peak (J. M. Scott unpublished). So far, no field assessment of the mortality of bristlecone pine has been completed on Schultz Peak and this is clearly needed because bristlecone pine is considered a protected species in Arizona under the Arizona Native Plant Law (ANPL) due to its rarity in the state (McDougal 1975). Not only is bristlecone pine rare in Arizona, but the dwarf mistletoe parasitizing it may also be threatened due to the recent fires and may need to be considered for protection under the ANPL. However, it should be emphasized again that the Flora of North America does not recognize subspecies *aristatae* (Nickrent 2016) and therefore, if followed prevents subsp. *aristatae* for consideration for possible protection under the ANPL. Based on our findings, *A. microcarpum* subsp. *aristatae* is morphologically distinct from subsp. *microcarpum* and should be considered as another rare plant present on the San Francisco Peaks that may be in need of protection (McDougal 1975; Rominger and Paulik 1983; Scott and Mathiasen 2009).

While the recent classifications of many previously described species of *Arceuthobium* into subspecies (Nickrent 2012, 2016) is an attempt that appears to be the simplification of species distinctions based primarily on molecular data, the practicality of doing this, in terms of recognition and the possible protection of currently described subspecies such as subsp. *aristatae*, is highly questionable. If the goal of plant systematics/taxonomy is to better understand the relationships among populations of *Arceuthobium* based on their morphology, phenology, and physiological (host affinity) differences, then obscuring these distinctions rather than recognizing them, diminishes rather than furthers our understanding of these ecologically and economically important parasitic plants. This study's results and those of related investigations (Mathiasen and Kenaley 2015, 2016, 2017) move this understanding forward.

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Table 1. Morphological measurements for *Arceuthobium campylopodum*, *A. microcarpum* subsp. *microcarpum*, and *A. microcarpum* subsp. *aristatae*. Data are listed as mean, (SD), [n]. Means followed by different capital letters in the same row were significantly different using a one-way analysis of variance (ANOVA) followed by a Tukey's honestly significant difference post hoc test ($\alpha = 0.05$). Mean measurements for *A. microcarpum* subsp. *aristatae* and subsp. *microcarpum* significantly different to *A. campylopodum* when compared via a Dunnett's test are in bold type ($\alpha = 0.05$; $P < 0.0001$). Lower case letters in the brackets designate sample sizes already listed in the same column. Plant heights are in cm and all other measurements are in mm. --------- indicates no data were collected.

Character(s)	***Arceuthobium campylopodum***	***Arceuthobium microcarpum* subsp. *aristatae***	***Arceuthobium microcarpum* subsp. *microcarpum***
Plant height			
Female	10.4 A (2.7) [600a]	**3.6** C (2.2) [177]	**6.9** B (1.3) [473]
Male	9.7 A (3.0) [a]	**2.7** C (2.0) [152]	**6.0** B (1.3) [383]
Basal diameter			
Female	3.4 A (0.7) [a]	**1.8** C (0.8) [167]	**2.0** B (0.6) [433]
Male	3.2 A (0.6) [a]	**1.8** B (0.5) [121]	**1.9** B (0.5) [257]
Third internode length			
Female	13.0 A (3.1) [a]	-------------------	**11.4** B (2.8) [120a]
Male	12.0 A (3.3) [a]	-------------------	**9.8** B (2.5) [100b]
Third Internode Width			
Female	2.5 A (0.4) [a]	-------------------	**1.7** B (0.2) [a]
Male	2.5 A (0.4) [a]	-------------------	**1.7** B (0.2) [b]
Flower Diameter	3.1 A (0.4) [400]	**2.4** B (0.5) [257]	**2.4** B (0.3) [355]
3-merous			
4-merous	4.2 A (0.5) [360]	**3.2** B (0.3) [30]	**3.2** B (0.7) [50]
Petal lobe length	1.6 A (0.2) [760b]	**1.3** B (0.2) [94a]	**1.3** B (0.2) [261c]
Petal lobe width	1.4 A (0.2) [b]	**1.1** B (0.2) [a]	**1.1** B (0.2) [c]
Anther diameter	0.6 A (0.1) [b]	**0.5** B (0.1) [a]	**0.5** B (0.1) [c]
Anther distance to tip	0.6 A (0.1) [b]	**0.5** B (0.1) [a]	**0.5** B (0.1) [c]
Fruit length	5.4 A (0.5) [480c]	**3.3** C (0.5) [281b]	**3.5** B (0.6) [530d]
Fruit width	3.7 A (0.4) [c]	**2.1** C (0.4) [b]	**2.2** B (0.4) [d]
Seed length	3.5 A (0.4) [c]	**2.4** B (0.3) [107c]	**2.4** B (0.3) [314e]
Seed width	1.5 A (0.2) [c]	**1.1** C (0.2) [c]	**1.1** B (0.1) [e]

Table 2. Summary of the principal characters separating *Arceuthobium campylopodum*, *A. microcarpum* subsp. *microcarpum*, and *A. microcarpum* subsp. *aristatae*. Data for morphological characters are means; plant heights in cm and all other measurements in mm. Numbers and hosts in bold type represent key morphological or physiological differences between the taxa. Host susceptibility classification based on information in Mathiasen and Hawksworth (1980), Hawksworth and Wiens (1996), and Scott and Mathiasen (2009).

Character	***Arceuthobium campylopodum***	***Arceuthobium microcarpum* subsp. *aristatae***	***Arceuthobium microcarpum* subsp. *microcarpum***
Plant height			
Female	**10.4**	**3.6**	**6.8**
Male	**9.7**	**2.7**	**5.9**
Basal diameter			
Female	**3.4**	**1.8**	**2**
Male	**3.2**	**1.8**	**1.9**
Flower diameter			
3-merous	**3.1**	2.4	2.4
Fruit length	**5.4**	3.3	3.4
Fruit width	**3.7**	2.1	2.2
Host Susceptibility			
Principal	***Pinus jeffreyi*** ***P. ponderosa***	***Pinus aristata***	***Picea engelmannii*** ***Picea pungens***
Secondary	*P. attenuata* *P. coulteri*	None	None
Occasional	*P. contorta* var. *murrayana* and var. *latifolia* *P. sabinianna*	***Picea engelmannii***	None
Rare		*Pinus strobiformis,* *Abies lasiocarpa* var. *arizonica*	*Abies lasiocarpa* var. *arizonica*

Table 3. Predicted taxonomic membership according to forward, stepwise discriminant function analyses (DFA) for the morphological classification of female (n= 6 characters) and male plants (n= 7 characters) using complete data. Anther diameter (AD); anther distance from tip (ADT); basal diameter (BA); fruit length (FL); fruit width (FW); petal length (PL); petal width (PW); plant height (PH); 3-merous flower diameter (3-FD). Sample size (n; female, male plants): *Arceuthobium campylopodum* (480, 402), *A. microcarpum* (314, 247), and subsp. *aristatae* (107, 94).

Stepwise DFA (step [character])	**Classified correctly to taxon membership** (%, [N predicted/ N field determined])			
	Total	*A. campylopodum*	*A. microcarpum* subsp. *aristatae*	*A. microcarpum* subsp. *microcarpum*
Female				
1. [FL]	84.2 [759/901]	98.8 [474/480]	23.4 [25/107]	82.8 [260/314]
2 [*], [PH]	94.0 [847/901]	99.2 [476/480]	98.1 [105/107]	84.7 [266/314]
3 [*], [*], [SL]	95.3 [859/901]	100.0 [480/480]	98.1 [105/107]	84.7 [274/314]
4 [*], [*], [*], [BD]	95.7 [862/901]	100.0 [480/480]	98.1 [105/107]	88.2 [277/314]
5 [*], [*], [*], [*], [SW]	96.0 [865/901]	100.0 [480/480]	98.1 [105/107]	89.2 [280/314]
6 [*], [*], [*], [*], [*],[FW]	96.1 [866/901]	100.0 [480/480]	96.3 [103/107]	90.1 [283/314]
Male				
1 [BD]	65.3 [492/753]	91.5 [368/402]	80.9 [76/94]	18.7 [48/257]
2 [*], [3-FD]	71.8 [541/753]	95.3 [383/402]	58.5 [55/94]	40.1 [103/257]
3 [*, [*], [PH]	86.6 [652/753]	95.0 [382/402]	89.4 [84/94]	72.4 [186/257]
4 [*], [*], [*], [PW]	87.5 [659/753]	95.8 [385/402]	89.4 [84/94]	73.9 [190/257]
5 [*], [*], [*], [*], [PL]	88.0 [663/753]	96.0 [386/402]	89.4 [84/94]	75.1 [193/257]
6 [*], [*], [*], [*], [*], [ADT]	88.6 [667/753]	96.8 [389/402]	89.4 [84/94]	75.5 [194/257]
7 [*], [*], [*], [*], [*], [*], [AD]	89.1 [671/753]	97.3 [391/402]	88.3 [83/94]	76.7 [197/257]

Table 4. Quadratic discriminant function (DFA) using complete morphological records and equal prior probability per taxon (0.333): assignment of field diagnosed female and male plants of *Arceuthobium campylopodum*, *A. microcarpum* subsp. *aristatae*, and *A. microcarpum* subsp. *microcarpum* to predicted taxonomic membership based on 6 female and 7 male characters (full-model for each sex).

Plant sex / *Arceuthobium* taxon (Total N = field determined plants)	**Assigned species membership according to DFA (%)** **[N= field determined plants]**		
	A. campylopodum	*A. microcarpum* subsp. *aristatae*	*A. microcarpum* subsp. *microcarpum*
Female			
A. campylopodum (480)	**100.0 [480]**	0.0 [0]	0.0 [0]
A. microcarpum subsp. *aristatae* (107)	1.0 [3]	**96.3 [103]**	2.8 [3]
A. microcarpum subsp. *microcarpum* (314)	0.9 [1]	8.9 [28]	**90.1 [283]**
Male			
A. campylopodum (402)	**97.3 [391]**	2.2 [9]	0.5 [2]
A. microcarpum subsp. *microcarpum* (257)	2.7 [7]	**88.3 [83]**	10.6 [10]
A. microcarpum subsp. *aristatae* (94)	1.1 [1]	20.6 [53]	**76.7 [197]**

Table 5. Canonical statistics: quadratic discriminant function analysis (DFA) of female (n= 6 morphological characters) and male plants (n= 7 morphological characters) using complete data or randomly selected records (n= 50 complete records/taxon) for *Arceuthobium campylopodum*, *A. microcarpum* subsp. *aristatae* and *A. microcarpum* subsp. *microcarpum*.

Canonical	**Eigenvalue**	**Percentage**	**Cumulative percentage**	**Canonical correlation**	**Likelihood Ratio**	**Approx. F**	***P*-value**
Female - Complete							
1	7.99	97.6	97.6	0.9427	0.0928	$F_{12, 1786}$= 339.68	<.0001
2	0.20	2.4	100.0	0.4074	0.8340	$F_{5, 894}$= 35.60	<.0001
Female - Resampled							
1	11.42	93.4	93.4	0.9589	0.0446	$F_{12, 284}$= 88.38	<.0001
2	0.80	6.6	100.0	0.6677	0.5542	$F_{5, 143}$= 23.00	<.0001
Male - Complete							
1	3.17	93.6	93.6	0.8717	0.1975	$F_{14, 1488}$= 132.88	<.0001
2	0.22	6.4	100.0	0.4212	0.8226	$F_{6, 745}$= 26.78	<.0001
Male - Resampled							
1	4.10	88.4	88.4	0.8966	0.1275	$F_{14, 282}$= 36.28	<.0001
2	0.54	11.6	100.0	0.5914	0.6502	$F_{6, 142}$= 12.73	<.0001

Table 6. Quadratic discriminant function analyses (DFA) of male and female plants using complete data for *Arceuthobium campylopodum*, *A. microcarpum* subsp. *aristatae*, and *A. microcarpum* subsp. *microcarpum*. Comparison of morphological characters (means) according to predicted classification to taxonomic membership. Ninety-five percent confidence intervals (±) were computed for comparison of mean differences. Mean plant heights in cm and all other measurements in mm.

Sex / Character(s)		***Arceuthobium campylopodum***	***Arceuthobium microcarpum* subsp. *aristatae***	***Arceuthobium microcarpum* subsp. *microcarpum***
Female				
	Plant height (PH)	10.3 (±0.2)	3.0 (±0.1)	6.7 (±0.2)
	Basal diameter (BD)	3.4 (±0.2)	1.6 (±0.1)	2.0 (±0.1)
	Fruit length (FL)	5.4 (±0..0)	3.2 (±0.1)	3.3 (±0.1)
	Fruit width (FW)	3.7 (±0.0)	2.1 (±0.1)	2.2 (±0.0)
	Seed length (SL)	3.5 (±0.0)	2.4 (±0.0)	2.4 (±0.0)
	Seed width (SW)	1.5 (±0.0)	1.1 (±0.0)	1.1 (±0.0)
Male				
	Plant height (PH)	9.5 (±0.3)	3.0 (±0.2)	7.0 (±0.3)
	Basal diameter (BA)	3.2 (±0.1)	1.8 (±0.1)	2.0 (±0.1)
	Flower diameter (3-lobed, 3-FD)	3.1 (±0.0)	2.4 (±0.1)	2.3 (±0.0)
	Petal length (PL)	1.5 (±0.0)	1.3 (±0.0)	1.3 (±0..0)
	Petal width (PW)	1.4 (±0.0)	1.1 (±0.0)	1.1 (±0.0)
	Anther diameter (AD)	0.6 (±0.0)	0.5 (±0.0)	0.5 (±0.0)
	Anther distance from tip (ADT)	0.6 (±0.0)	0.5 (±0.0)	0.5 (±0.0)

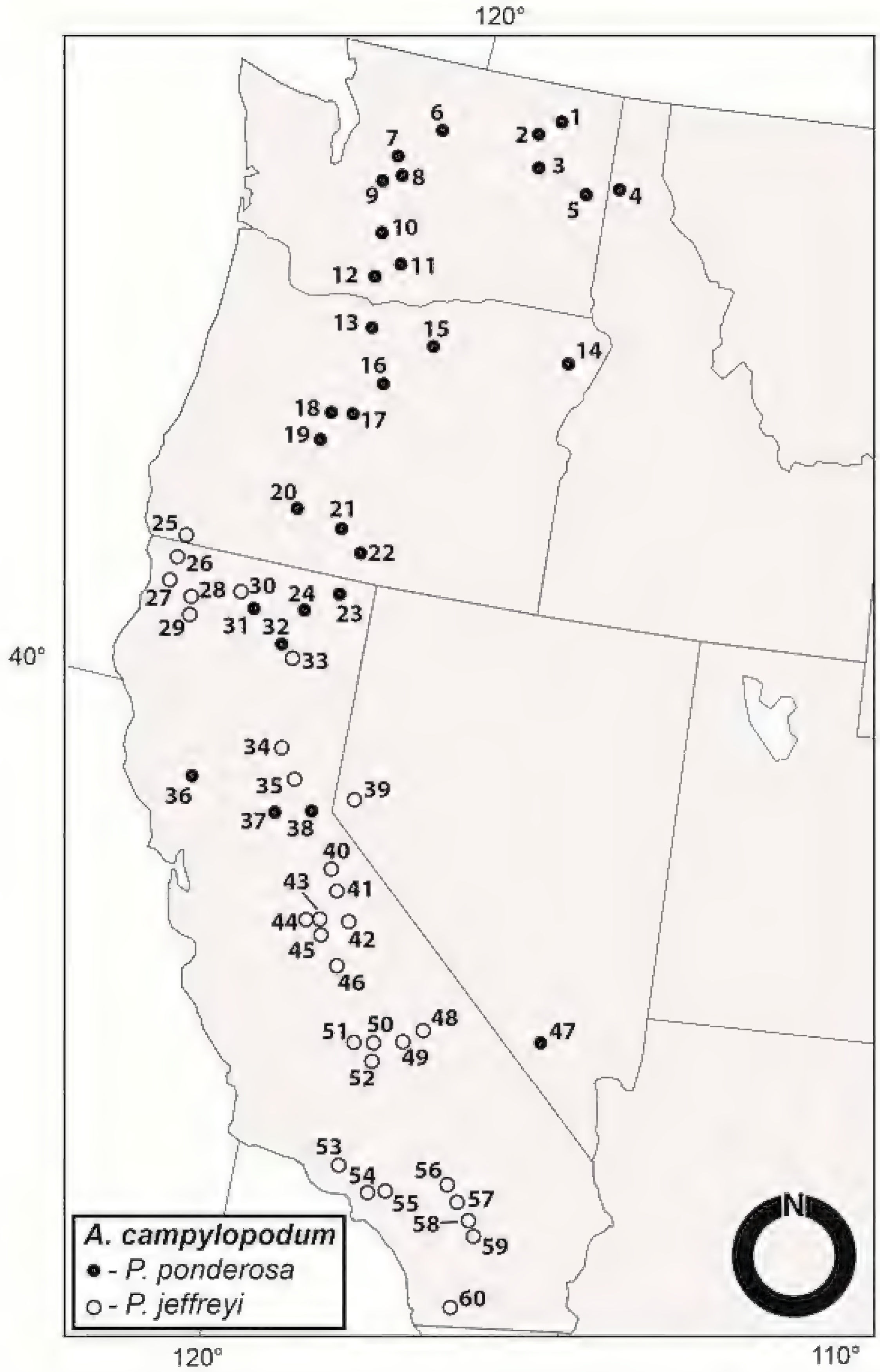


Figure 1. Approximate locations of collection sites for *Arceuthobium campylopodum* in Washington, Idaho, Oregon, California, and Nevada. Closed circles present locations where plants were collected from *Pinus ponderosa*. Open circles represent locations where plants were collected from *P. jeffreyi*. Numbers correspond to the following locations: **Washington**: **1**- 4.5 km N of Gifford on St. Rte. 25, **2** - 20 km S of Fruitland on St. Rte. 25, **3** - 2 km NW of Nespelem on St. Rte. 155, **5** - 16 km S of Spokane on St. Rte. 195, **6** - 2.5 km W of St. Rte. 153 on Squaw Creek rd., **7** - Lake Wenatchee on Chiwawa River Loop rd., **8** - 2.6 km W of Squilchuck St. Park on road to Mission Ridge Ski Area, **9** - 0.8 km W of St. Rte. 97 on St. Rte 970, **10** - 17.6 km E of White Pass on St. Rte. 12, **11** - 2 km N of Satus Pass on St. Rte. 97, **12** - 3 km S of Trout Lake on St. Rte. 141; **Idaho**: **4** - 2.3 km N of Coeur d'Alene on Fernan Lake rd.; **Oregon**: **13** - 6.4 km W of Friend on forest rd. 27, **14** - 6.4 km S of Joseph on E shore of Wallowa Lk., **15** - 9.4 km on Sheep Cr. rd from forest rd. 51, Wallowa-Whitman Nat. For., **16** - 1.8 km E of Ochoco Summit on St. Rte. 26, **17** - 12.2 km W of St. Rte. 97 on St. Rte. 138, **18** - 15.2 km S of Sisters on forest rd. 16, **19** - 1 km from forest rd. 44 on forest rd. 4410, Pringle Falls Exp. For., **20** - Fort Klamath Cemetery on St. Rte. 62, **21** - 3 km W of Quartz Mtn. Pass on St. Rte. 140, **22** - Warner Mtn. Ski Hill on St. Rte. 26, **25** - 6 km S of Takilma on Greyback rd.; **California**: **23** - 3.4 km W of County rd. 48 on forest rd. 73, west shore of Goose Lk., **24** - 16 km N of Adin on St. Rte. 299/139, **26** - 1 km S of forest rd. 17N26 on forest rd. 17N11, Klamath Nat. For., **27** - 6.2 km W of St. Rte. 96 on Dillon Mtn. rd., **28** - 9.6 km S of Callahan on St. Rte. 3, **29** - 10 km E of St. Rte 3 on forest rd. 17, Shasta-Trinity Nat. For., **30** - 2.4 km W of Stewart Hot Springs on forest rd. 17, **31** - 2 km N of St. Rte. 89 on Mt. Shasta Ski Park rd., **32** - 0.1 km S of St. Rte. 299 on St. Rte. 89, **33** - 2 km S of Old Station on St. Rte. 44, **34** - 2 km W of St. Rte. 44 on forest rd. 101, **35** - 14.4 km W of Susanville on St. Rte. 36, **36** - 19.5 km N of Upper Lake on Pillsbury Lk. rd., **37** - 7.7 km N of Pollock Pines on forest rd. 4, **38** - at entrance to Sugar Pine State Park, west shore of Lk. Tahoe, **40** - 1 km N of Markleeville on St. Rte. 89, **41** - Silver Creek Campground on St. Rte. 4, **42** - Column of the Giants on St. Rte. 108, **43** - Pinecrest Transfer Station 0.5 km W of Pinecrest on St. Rte. 108, **44** - 1 km W of Long Barn on St. Rte. 108, **45** - 8.5 km E of Crane Flat on St. Rte. 120, **46** - 2 km W of Big Creek on rd. to Shaver Lk., **48** - 8.5 km W of Sherman Pass on forest rd. 22S05, **49** - 2.2 km S of Troy Mdws. Campground, Sequoia Nat. For., **50** - 5.8 km N of rd. to Johnsonville on Western Divide Highway, **51** - Pine Flat, Sequoia Nat. For., **52** - Tiger Flat, Sequoia Nat. For., **53** - 6.2 km S of St. Rte. 33 on rd. to Mt. Reyes, **54** - 1.4 km W of Cloud Burst on St. Rte. 2, **55** - 1 km W of Big Pines on St. Rte. 2, **56** - 2.4 km N of Fawnskin on forest rd. 2N71, **57** - 1.9 km from St. Rte. 38 on rd. to Jenks Lk., **58** - near Ranger Station in Idylwild, **59** - 1.1 km S of the S Fork San Jacinto River Bridge on St. Rte. 74, **60** - 0.5 km S of Horse Heaven Campground on Sunrise Highway; **Nevada**: **39** - Bowers Mansion St. Park, **47** - 4.1 km W of Ranger Station at Old Ski Tow Historic Site, Kyle Canyon.

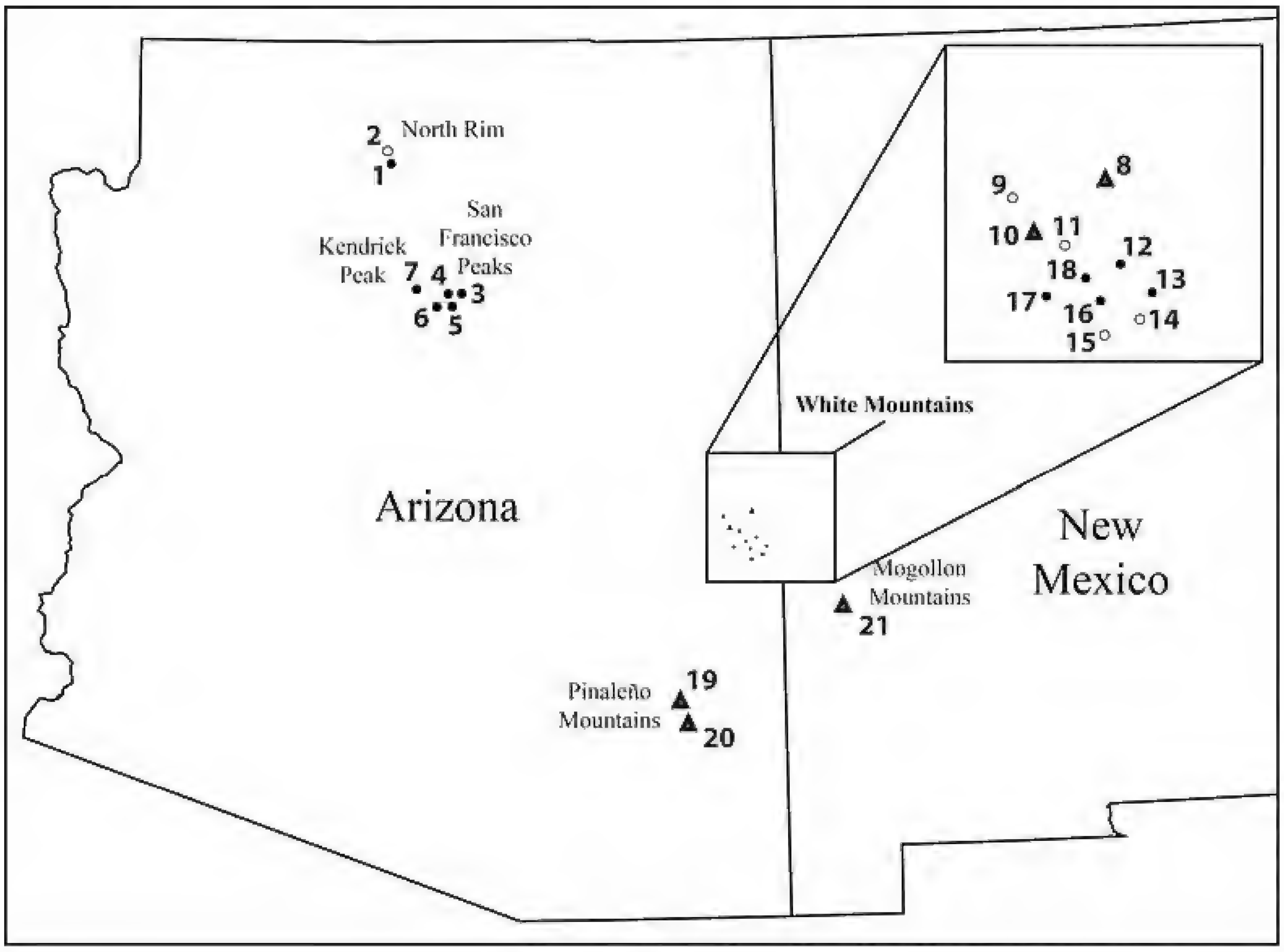
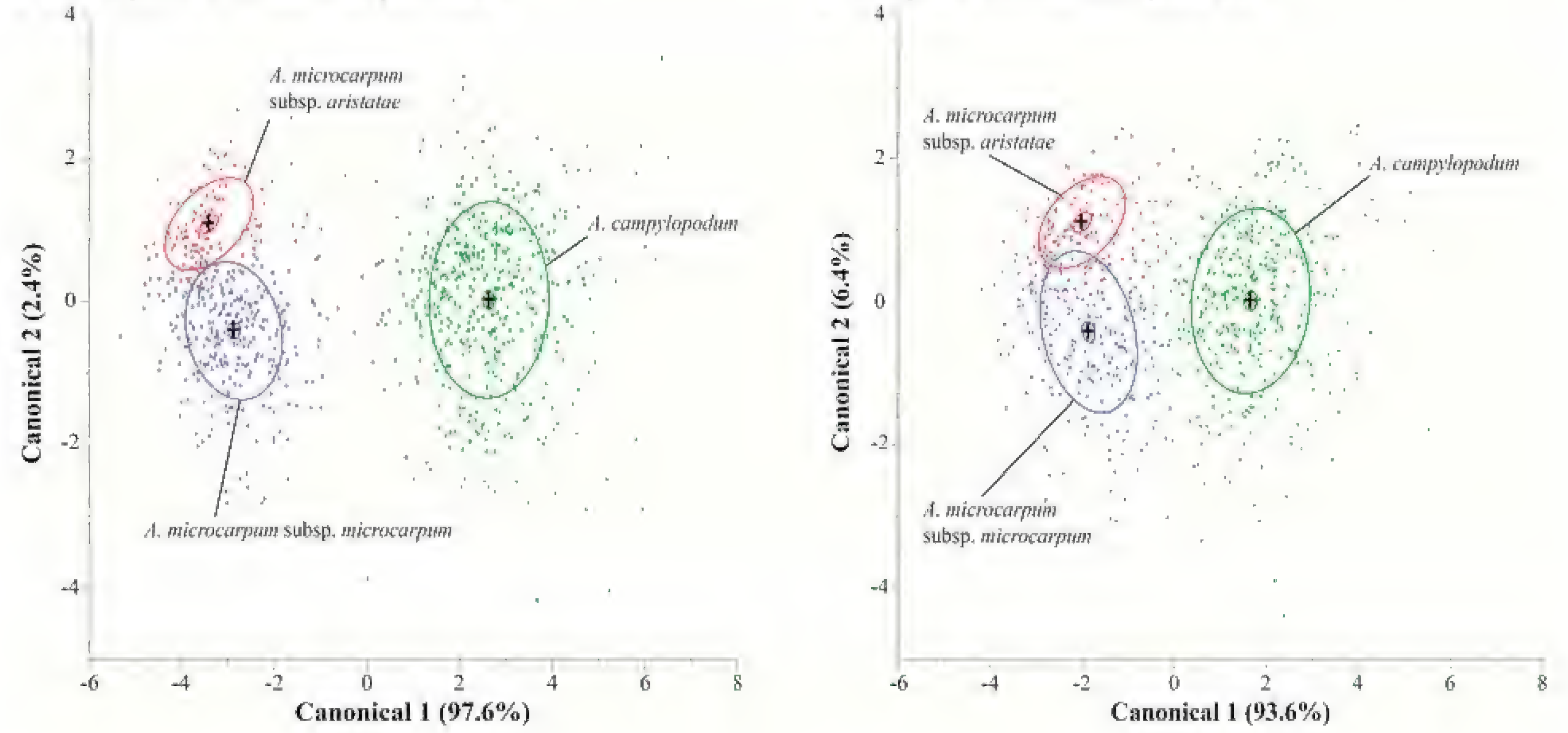
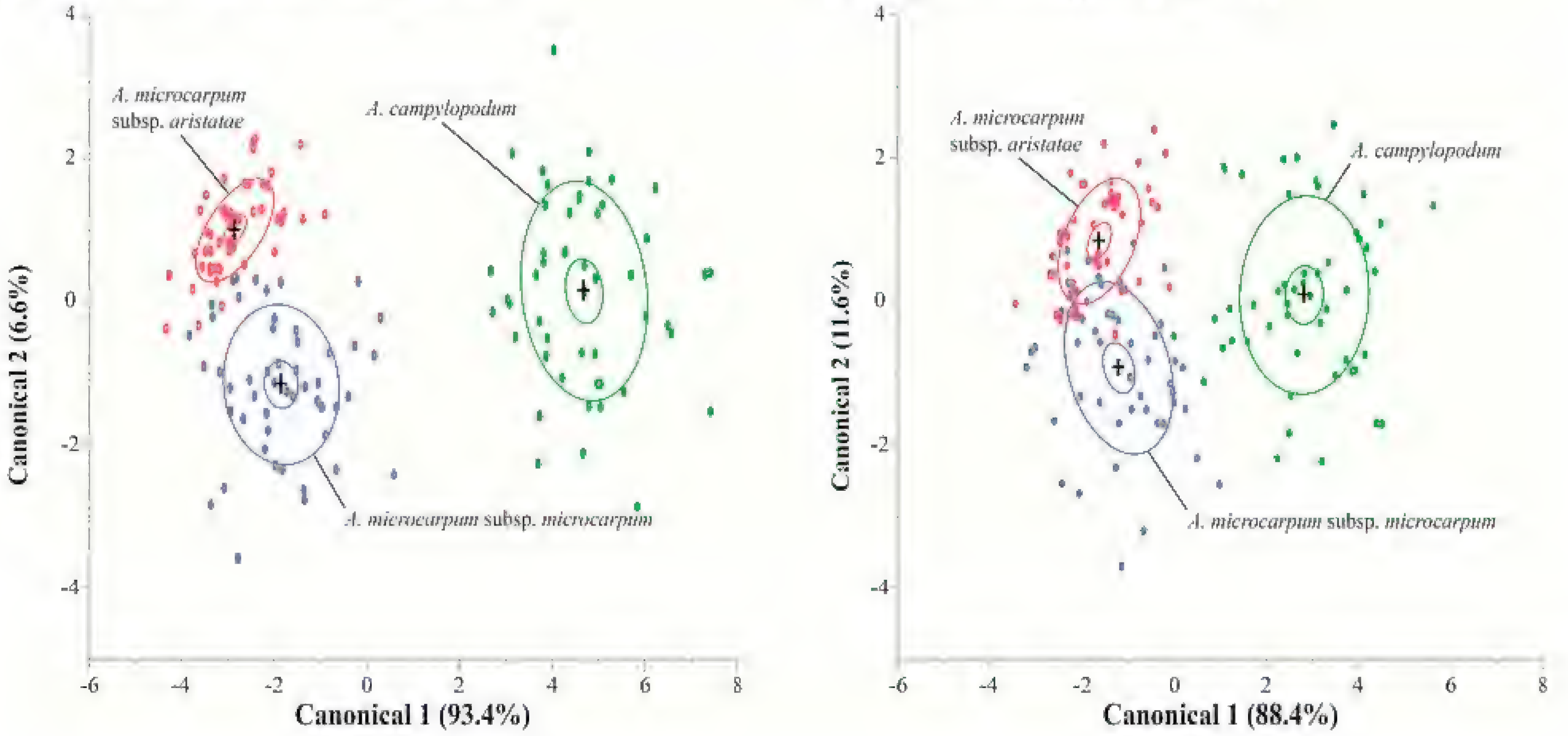


Figure 2. Approximate locations for plant collections of *Arceuthobium microcarpum* in 2007 and 2017. Collection sites for 2007 are indicated by closed circles. Collection sites where plants were sampled in 2007 and 2017 are indicated by open circles. Sites where plants were sampled in 2017 only are indicated by open triangles. Plants were collected from bristlecone pine (BCP), Engelmann spruce (ES), or blue spruce (BS) as indicated in parentheses for each collection site. **Arizona**: **1**- Point Royal Road, North Rim Grand Canyon National Park (BS), **2** - 6 km south of park boundary on State Route 67, North Rim Grand Canyon National Park (BS), **3** - Inner Basin of San Francisco Peaks (ES), **4** - "Secret Meadow" in southeast Inner Basin of San Francisco Peaks (BCP and ES), **5** - Schultz Peak BCP and ES), **6** - Weatherford Trail ca. 1 km south of Doyle Saddle (BCP), **7** - summit of Kendrick Peak (ES), **8** - 1 km W of Greer on forest road 575 (BS), **9** - Lee Valley Reservoir (BS), **10** - Big Lake at junction of forest road 249 and State Route 261 (BS), **11** - 4 km east of Big Lake on forest road 249 (BS), **12** - Williams Valley near forest road 249 (BS), **13** - 6 km S of Alpine near State Route 191 (BS), **14** - 3 km N of Hannagan Meadows near State Route 191 (BS), **15** - Cache Cienega near forest road 26 (ES and BS), **16** - along forest road 72 (ES and BS), **17** - along forest road 402 (ES and BS), **18** - John's Canyon near forest road 405 (BS), **19** - Soldier Creek campground (ES), **20** - Hospital Flat campground (ES); **New Mexico**: **21** - Willow Creek campground.



A. Female plants: full-model, complete data.

B. Male plants: full-model, complete data.



C. Female plants: full-model, resampled data.

D. Male plants: full-model, resampled data.

Figure 3. Canonical plots for discriminant function analyses (DFA) of *Arceuthobium campylopodum*, *A. microcarpum* subsp. *microcarpum*, and *A. microcarpum* subsp. *aristatae* based on morphological characteristics of female (A, C) and male plants (B, D) shown in Table 6. Multivariate means (squares) were computed using complete data for each species by sex (A, B), whereas, to further validate the DFA, means were also calculated using a random subset (50 complete records/taxon) of female (C) and male plants (D), respectively. For each taxon (A-D), the inner ellipse correspond to a 95% confidence limit for the mean, and the outer ellipse represent a normal 50% contour illustrating the approximate area within which 50% of plants for each species reside.

Photosynthetic Comparison of *Carex planostachys* (C_3) and *Aristida purpurea* (C_4) to Light and Water Manipulations

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ABSTRACT

In some southwestern *Juniperus* woodlands, *Carex planostachys* (C_3 sedge) occurs at high cover below the canopy and *Aristida purpurea* (C_4 grass) has high cover in associated grasslands or gaps. Availability of light or water are often suggested causing this variation. Photosynthetic measurements were made as light and water were manipulated in a transplant field study. Monocultures of potted plants were placed below canopy and in an associated gap for three summer months. There were significant main experimental effects on photosynthetic and transpiration rates including location, shade, water level and species. Both species were capable of photosynthetic gain in either location, but CO_2 uptake was three and one-half times greater for the grass in full light. Transpiration rates for both species mostly tracked photosynthetic rates. Examining means plots of three-way interactions showed that no supplemental water had a small effect on *A. purpurea* photosynthetic rates below the canopy, but a large reduction in the gaps. Rates were opposite indicating an interaction. For *C. planostachys* in either location non-watering had limited negative effects. *Aristida purpurea* could carry out photosynthesis at intermediate light levels below the canopy but at reduced rates with no interaction. *Carex planostachys* photosynthesis increased below the canopy when shaded, but decreased without shade in the gaps, an interaction. Water addition impacted photosynthetic rates of both species, but the greatest effect was on *A. purpurea* in the gaps. Light levels and water levels interact to regulate the presence of these two species in *Juniperus* woodlands and associated canopy gaps, they do not act independently. Published on-line www.phytologia.org *Phytologia 100(1): 91-103 (Mar 16, 2018).* ISSN 030319430.

Key Words: A_{net}, E_{leaf}, *Juniperus* woodlands, canopy, gap, drought, sedge, grass

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Juniperus communities occur throughout much of the U.S. in semi-arid habitats between the deserts or arid grasslands and mountain forests (Van Auken 2000; Van Auken and McKinley 2008). These communities have 17-80 cm/y rainfall, little summer rain and high rainfall variability with frequent droughts (World Climate 2011). Encroachment of *Juniperus* into many grasslands has created a matrix dominated by woodlands with small associated intercanopy grassland gaps and an overall reduction in grass cover (Reid et al. 1999; West 1999; Archer et al. 2001; Briggs et al. 2002; Van Auken and Smeins 2008). For many communities, the patterns of plant distributions results from the environmental gradients occurring across the habitat, the severity of stressors, and spatial variation in soil type and depth (Givnish 1988; Holmgren et al. 1997; Brady and Weil 2002; Schwinning et al. 2002; Brooker et al. 2008; McDowell et al. 2008; Keddy 2017). Causes for reductions in grass cover following *Juniperus* encroachment are speculative; however, light attenuation by the evergreen *Juniperus* canopy is suspeced as a leading agent of change (McPherson et al. 1991). Some plants can adapt to a light regime, thus they are not restricted to a high light or low light environment (Givnish et al. 2004). C_4 grasses typically require full sun in order to maintain high photosynthetic rates (Gehring and Bragg 1992; Knapp and Medina1999; Turner and Knapp 1996; Wayne and Van Auken 2012; Keddy 2017), but some have a plastic response (Naumburg et al. 2001). Additional controlling abiotic factors could include amount and seasonality of soil water, temperature and the concentration of other soil nutrients (Wayne and Van Auken 2004; Breshears 2008,) and the factors may act singularly or synergistically to impact plant distributions (Fowler 1990).

During the past 150 – 200 years, *J. ashei* woodlands have encroached throughout much of the central Texas Edwards Plateau. This area was previously mostly a grassland and southern extension of the Great Plains (Diamond and Smeins 1985). In *J. ashei* communities woody cover and grass cover seem inversely related, but the influence of the canopy rapidly declines at the canopy edge (Fuhlendorf et al. 1997; Wayne and Van Auken 2004). Thus, grass cover is high away from the canopy, reduced at the canopy edge and eliminated below the canopy. The idea that *Juniperus* encroachment results in the elimination of most, if not all, herbaceous cover is not always correct and some C_3 sedges increase in cover below the canopy (Gehring and Bragg 1992; Wayne and Van Auken 2004). While the light level below the *J. ashei* canopy is a major controlling influence on the reduced grass cover and biomass below the canopy (Wayne and Van Auken 2004; Young and Bush 2009), a C_3 sedge, *Carex planostachys* (cedar sedge), in many cases has high cover (Wayne and Van Auken 2008). However, at the canopy edge and in the gaps, C_3 and C_4 grasses provide the main cover (Fowler 1990; Wayne and Van Auken 2008).

A second principle factor affecting plant distribution, survival, and physiological function in these arid and semi-arid regions is water availability. In general, drought reduces available water and affects transpiration rates, which has a negative impact on photosynthesis (Tardieu and Simmoneau 1998; Sperry et al. 2003; Keddy 2017), resulting in loss of productivity. Isohydric plants have tight regulation of stomata that limits declines in water stress through reduced transpiration. In severe drought, isohydric plants experience hydraulic loss with infrequent recovery thus limiting their distribution (McDowell 2008). Anisohydric plants have limited stomatal control, are resistant to hydraulic loss and are likely to occur in drought prone areas (Maseda and Fernández 2006; McDowell et al. 2008; McDowell 2011). For example, during drought, some *Juniperus* stems can reach water stress of -10 MPa while maintaining hydraulic conductivity and continued transpiration (Pockman and Sperry 2000; Wayne and Van Auken 2002). Non-woody species typically cavitate (develop gas bubbles in the xylem) at water stress below -3.0 MPa, but maintain some stomatal control to prevent catastrophic water loss (Busch 2001; Sperry et al. 2003; Stiller et al. 2003). C_3 and C_4 grasses may exhibit either isohydric or anisohydric responses, to short-term drought (Tardieu and Simmoneau 1998; McDowell et al. 2008: McDowell 2011).

The causes for distributional differences among grasses and grass like plants in *J. ashei* woodlands are mostly speculative, but are considered related to water and or light availability (Wayne and Van Auken 2012). *Carex planostachys* displays an anisohydric response and appears well adapted to a reduced light environment, but is tolerant of high light levels occurring in gaps (Wayne and Van Auken 2009; 2012). Cover for *C. planostachys* is high in areas below the canopy and at the canopy edge, but at lower cover values in gaps (Wayne and Van Auken 2008). C_4 grasses have high photosynthetic rates in grasslands where canopy imposed light attenuation does not occure (Knapp and Medina 1999; McCarron and Knapp 2001; Fay et al. 2002; Wayne and Van Auken 2012). *Aristida purpurea* occurs in the grasslands associated with *J. ashei* in high light habitats (Fowler 1990; Wayne and Van Auken 2008). Many studies of grass in prairie systems suggest that an overhead canopy is inhibitive to grass growth, either through light attenuation (Fowler 1990; Briggs et al. 2002), allelopathy or limited water (McDowell et al. 2008; Young and Bush 2009; Wayne and Van Auken 2012).

PURPOSE

The purpose of this study was to examine the importance of light and water to the distribution of a C_3 sedge *C. planostachys* found mainly below a *J. ashei* canopy and a C_4 grass *A. purpurea*, found mainly in an associated intercanopy gap.

MATERIALS AND METHODS

Study site and species description

This was a transplant study using monocultures of two soecies of potted plants to test the influence of light and water on leaf level photosynthesis and transpiration at two locations. The study site (ca. 2000 m^2) was located on the University of Texas at San Antonio Texas west campus on the southern edge of the Balcones Escarpment of the Edwards Plateau of central Texas. The area consists of closed canopy *J. ashei* woodlands and associated intercanopy gaps. The woodland vegetation is predominately *J. ashei* and *Quercus virginiana* (Van Auken 1988; Grunstra and Van Auken 2015). The intercanopy gaps include various perennial C_4 grasses and a number of C_3 herbaceous annuals (Terletzky and Van Auken 1996). *Carex planostachys* occurs along a gradient from below the woodland into the gap (Wayne and Van Auken 2008). Supplemental treatments were applied with four combinations of water and shade: water and shade (+S, +W), shade only (+S, -W), water only (-S, +W) and no water and no shade (-S, -W). The species used for this study were *C. planostachys* Kunze (cedar sedge) and *Aristida purpurea Nutt. Var. longiseta* (Steud.) *Vasey* (*Fendler threeawn*) (USDA, NCRS 2017, hereafter *Aristida purpurea* or *Aristida*).

Carex planostachys is a low growing herbaceous perennial with culms 4 – 30 cm long. It is reported mostly in arid areas on thin limestone derived soils of the Edwards Plateau, in north central Texas into Oklahoma, western areas of the Trans-Pecos mountains and south toward the Rio Grande Plains (Correll and Johnston 1979; USDA, NCRS 2017). *Aristida purpurea* is a tufted perennial C_4 grass with culms 15 – 35 cm and leaf blades 4 – 10 cm long. It is reported on rock or sandy slopes of the Trans-Pecos, Edwards Plateau and South Texas plains to North Dakota, Washington State and south into Mexico (Correll and Johnston 1979; USDA, NCRS 2017).

Transplant procedures, site preparation and treatment design

Plastic pots, 15 X 15 cm, were lined with Zip-Loc™ bags and filled with ca. 1400 g of a dried, sieved, low nutrient, Patrick series soils. Individual raments of *C. planostachys* were obtained on campus below the *J. ashei* woodland in March 2005 and transplanted into prepared pots. Roots were trimmed to 15 cm prior to transplanting. Seeds of *A. purpurea* (Native American Seed, Junction, TX) were planted into additional prepared pots. All pots were initially watered with 500 ml of deionized water and 150 ml of a 25 % fertilizer solution (Miracle-Gro®). To start, pots were kept in a temperature-controlled greenhouse (37° C) and watered as needed to maintain the soil at field capacity.

After germination of *A. purpurea* seeds, plants were thinned to 5 plants per pot. In April 2005, two benches with clear plastic roof panels to exclude rainfall were set up outside the greenhouse to serve as an intermediate site to acclimate plants to outside conditions. In addition, one bench was covered with neutral density shade cloth (75 % occlusion) to reduce light levels (PPF – photosynthetic photon flux) to values similar to those occurring below the *J. ashei* canopy (approximately 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$, Wayne and Van Auken 2004; Wayne and Van Auken 2008). Light reduction was verified with a LiCor (Lincoln, NE) LI-190 quantum sensor (400-700 nm). All pots were moved outside, with *C. planostachys* on the shaded bench and *A. purpurea* on the unshaded one and provided a second application of 150 ml of a 25 % fertilizer solution. On June 1 2005, pots were randomly assigned to a location and treatment. Prior to establishing pots in the field, the canopy site was cleared of understory woody plants and overstory branches were manipulated with ropes to create a continuous canopy. In the gap, an unshaded site was prepared by removing woody plants and mowing the grass. Pots were given a final application of fertilizer (150 ml of a 25 % solution) and placed into the field.

Below the canopy, individual blocks were placed randomly around a *J. ashei* tree (n = 5 trees), between 0.5 and 1.0 m from the trunk. In the gap, blocks were arranged randomly in rows, perpendicular to the solar track, ca. 5 m apart and pots within an individual block were randomly placed ca. 1 m apart.

Supplemental shade was applied with 75 % shade cloth shaped into cones that were 70 cm in height and 50 cm diameter. Shade cones were placed over shade treatment pots and secured to the ground with nails. All pots were checked to ensure a moist surface and marked with labeled flags, either red or blue, for ease in identification of block and treatment. Watering of the no water treatment was discontinued on this day. Plants were monitored every three days and water was added to the water treatment to maintain field capacity, ca. 150 ml for each application.

Physiologic measurements

Photosynthetic measurements were made in late August on fully expanded leaves with a LiCor 6400 portable photosynthetic meter (LiCor, Lincoln, NE) with an integrated light source (measurements made in July, were consistent with August measurements, but not presented). Ambient light levels were determined on a cloudless day to establish global light values for each location with and without supplemental shade. Photosynthetic light was applied with an integrated red-blue LED light source at predetermined global values. Below the canopy, photosynthetic photon flux (PPF) was 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$, below the canopy with shade PPF was 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$, in the gaps the PPF was 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and with shade it was 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Measurements for photosynthesis (A_{net} – $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) and transpiration (E – mol $H_2O \cdot m^{-2} s^{-1}$) were made between 1000 – 1200 hr, after vapor pressure deficit stabilized. Chamber temperature and humidity were held at initial ambient levels, with values established prior to the first measurement. Three to 5 leaves per plant were used, to maximize leaf area, and they were placed into the chamber cuvette and photosynthetic measurements were logged after a stable coefficient of variation (< 0.2 %) was reached, approximately 2 to 3 minutes per plant. Because of the time involved for an individual plant response, it was necessary to conduct measurements over two consecutive days.

Analysis

The experimental design was a randomized block design with replications (n = 5 blocks), with measurement made in late August. Nested within each block were 2 locations (L). Within each location were 2 species (SP), 2 levels of shade (S) and 2 levels of water (W). Treatments combinations for each species were supplemental shade and supplemental water (S+ W+), supplemental shade and no water (S+ W-), no shade and supplemental water (S- W+) and no shade and no water (S- W-). To test for overall effects, data were analyzed with ANOVA in a mixed model design (SAS 2005) with location nested in blocks, and species, shade and water treated as random effects. For interactions Bonferroni corrections were used to determine where significant differences occurred between location, added shade, added water, and species.

RESULTS

Photosynthesis results for August measurements are presented, but only A_{net} photosynthesis and transpiration E_{leaf} . Net photosynthesis varied significantly by location, shade, water, and species (Table 1), with six significant two-way interactions (S x W, S x SP, W x Sp, L x S, L x W, and L x Sp) and two significant three-way interactions (L x S x W and L x S x Sp). Greatest A_{net} was in the gaps (L), with no shade, but with added water (Figure 1a). *Aristida* had an A_{net} about 3.5 times that of *Carex* (Figure 1a). Transpiration varied significantly by location, shade, and water (Table 1), and there were two significant two-way interactions (L x S and L x W) and two significant three-way interactions (L x S x W and L x S x Sp). Main effects for transpiration were location (highest in gaps), shade (lowest with added shade), and water (greatest when added), with the rate for *Aristida* higher than that of *Carex* (Figure 1b).

For the most part, A_{net} is greatest in the gaps (location) and lower below the canopy, but this is coupled with additional shade having a general suppression of A_{net} which is linked to added water and the species. Because the three-way interactions (L x S x W and L x S x Sp, Table 1) were significant, shade and water effects should be examined and compared simultaneously per species. Thus we used means plots to show the effects of shading (+ and -) and water additions (+ and -) (Figure 2).

For *Aristida,* greatest A_{net} was in the gaps with added water (Figure 2 top-left). Below the canopy when watered, photosynthesis was reduced. Without added water and below the canopy photosynthesis did not change very much, but in the gaps it was reduced to about 6 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. Note that the lines connection the gap treatments and the canopy treatments cross indicating the significant interaction between treatments. When light treatments were examined, the shade+ treatments were lower than the shade– treatments, but the lines for the gap and canopy were parallel indicating no significant interaction.

For *Carex* when water was added canopy and gap photosynthetic values were very similar (Figure 2, top right). When plants were not watered, rates were the same below the canopy or in the gaps, reduced to about 3 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (Figure 2, top right). When light levels were examined, lowest A_{net} was below the canopy, with added shade (Figure 2, lower right). When shade was removed, *Carex* greatest A_{net} was below the canopy and lowest in the gaps. Note the lines connection the gap and canopy cross indicating a signigicant interaction.

Transpiration rates are presented in figure 3 and are similar to photosynthetic rates. Transpiration rates for *Aristida* were largest in the gaps when water was added and lowest in the non-watered gap treatment (Figure 3, upper left). Transpiration rates for *Aristida* were also high below the canopy when water was added but lower without added water. Also note the connected gap and canopy lines cross indicating a significant interaction. When shading was examined, transpiration was the same below the canopy or in the gaps (Figure 3, lower left). When shade was removed, transpiration in the gaps was much higher than the rate below the canopy which changed very little, and an interaction is suggested.

For *Carex*, transpiration was highest in the gaps with added water and reduced without addition of water (Figure 3, upper right). Transpiration was lower below the canopy and lowest when water was not added and the connected lines for the gap and the canopy were parallel indicating no interaction. Examining shading for *Carex* showed that highest transpiration was in the gap treatment with no shading and lower in the shade treatment (Figure 3, lower right). When transpiration below the canopy was examined, shade reduced transpiration more than the no shade treatment but the connected lines for the gap and the canopy were parallel indicating no interaction.

DISCUSSION

Plants are found at different points along complex environmental gradients, and the factors that produce these gradients effect plant photosynthetic activity, growth, survival and distribution (McDowell 2011; Keddy 2017). Light and water availability simultaneously affect photosynthetic activity including transpiration or water loss (Chaves et al. 2003; Tuzet et al. 2003). Thus, they are coupled with both influencing carbon gain. Prolonged drought places greater survival risk on plants because of reduced photosynthate to meet metabolic demands (McDowell 2011). During this study light levels and water availability were manipulated below a *J. ashei* canopy and in adjacent intercanopy gaps or locations. Light levels, water availability, and location were significant in the experiment. The manipulation of these factors effected the responses of the two species differently. This led to significant three way interactions in the experiment making interpretation challenging to discern.

Reduced light levels reduced the photosynthetic rate of *Aristida purpurea*, but the canopy or added water moderated the response. This suggested that *Aristida* is a sun plant as reported earlier from measurements of *in situ* plants (Wayne and Van Auken 2004; 2008; 2009). Increased light levels in the gaps reduced the photosynthetic rate of *Carex planostachys* unless shade was added making the gap treatment light levels similar to the canopy light levels. Observations on these plants gradient position when cover and physiologic responses are considered suggest that *C. planostachys* is a shade plant and quite different in its response to light compared to *A. purpurea* (Wayne and Van Auken 2008; 2009; 2012).

Transpiration rates mostly tracked photosynthesis rates, being higher in the gaps, with or without added shade or added water. The *J. ashei* trees were responsible for the additional light attenuation of the canopy. Artificial shade in the gap had a similar effect to that of the woodland canopy. Below the canopy, supplemental shade was additive causing further reductions in carbon uptake in *Aristida.* These results were consistent with the expected response in light availability due to the canopy presence (Muth and Bazzaz 2002; Breshears 2008; Wayne and Van Auken 2012). Distinct gradients in light levels do occur in *J. ashei* woodlands and are related to location, from below the canopy into the gap. While light levels are intermediate at the canopy edge, the focus of this study was the influence of light levels in the closed canopy understory and the gap where the canopy was not present.

Carex planostachys is tolerant of deep shade, with a reported light compensation of ca. 4.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and light saturation of ca. 151 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Wayne and Van Auken 2009). The results from this study suggest the benefit of light reduction by the canopy to carbon gain by *C. planostachys*. This was apparent both below the canopy with and without supplemental shade and in the gap with supplemental shade. Peak photosynthetic rates were observed between 600 and 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but photosynthetic gain still occurred at reduced light levels (120 $\mu mol \cdot m^{-2} \cdot s^{-1}$), which is above the light compensation point (Wayne and Van Auken 2009; 2012).

Aristida purpurea, was expected to have higher photosynthetic rates with increasing light levels because it is a C_4 grass which typically have higher photosynthetic rates than C_3 plants (Keddy 2017). The photosynthetic rate of *A. purpurea* measured in the gaps at high light levels (ca. 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$), was expected to be about 20 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, but was half that value. Rates were probably lower because plants were in pots with limited soil and water availability. The photosynthetic response of *C. planostachys* was low, typical of shade plants, and what we have reported in the past (Wayne and Van Auken 2009). However, the rate below the canopy with added water suggested soil water reduction and competition with overstory *J. ashei* trees.

Plants can respond to abiotic or biotic factors to conserve water, maintain turgor, prevent catastrophic cavitation, and protect the photosystem (Tyree and Sperry 1989; Galmés et al. 2007; McDowell et al. 2008; McDowell 2011). An increase or decrease in photosynthesis results in a similar response of transpiration. Besides light as a primary regulator of the photosynthetic response, available soil water, temperature, and vapor pressure deficit have an influencing effect (Chaves et al. 2003; Keddy 2017). Despite ca. 60 days of drought in the current study, a reduction in available plant water appeared to have specific impacts on photosynthesis in both species. This is an expected response from anisohydric plants (McDowell et al. 2008; McDowell 2011). During stress photosynthesis should decrease and is related to water stress interfering with metabolic activity (Wong et al. 1979; Sharkey 2005; McDowell 2011). Enhanced transpiration is reported to occur in many plants while under severe stress, despite stomatal control, and provides some benefit through evaporative cooling, reducing leaf temperature to protect from damaging heat (Larcher 1995; Valladares and Pearcy 1997; Chaves et al. 2003). *Aristida purpurea*, a C_4 plant may avoid drought stress by maintaining smaller stomatal apertures reducing water loss (McCarron and Knapp 2001).

Differences between species photosynthetic responses were observed and were related to the light and water levels manipulated during the experiment. Other species have demonstrated similar responses (McDowell et al. 2008; McDowell 2011). In terms of functional groups, *C. planostachys* appears like a shade tolerant plant and *A. purpurea* as a shade intolerant or sun plant (Wayne and Van Auken 2009, 2012). This in part explains the high cover of *C. planostachys* reported below the J. ashei canopy and the high cover *A. purpurea* in adjacent gaps (Wayne and Van Auken 2004; 2008; 2009). Temperature differences between treatment locations, thought slight and not examined here, could help further delineate why the two species are found in different though adjacent habitats (Sharkey 2005). However, both species appeared to gain carbon in intermediate light habitats, which helps explains near equal cover of both species at the *J. ashei* canopy edge (Wayne and Van Auken 2008). Canopy moderation of environmental factors, favoring various herbaceous plants and woody seedlings has been reported for deciduous woodlands and some evergreen woodland (Hughes et al. 2006). In prairie systems, tall grasses have a similar effect to that of a woodland canopy reducing the stress on short C_3 and C_4 grass (McCarron and Knapp 2001; Fay et al. 2002; Fay et al. 2003).

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Table 1. ANOVA table testing for within significant main effects of location (L), shade (S), water (W), species (Sp) and all interactions. Parameters measured were leaf net photosynthesis (A_{net} – µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) and transpiration ($E_{leaf}$ – mmol $H_2O \cdot m^{-2} s^{-1}$). Measurements were made in late August 2005. *P*-values are given for effects included in the model. ns = not significant.

August	**A_{net}-*P***	**E_{leaf}-*P***
L	≤ 0.0001	≤ 0.0001
S	≤ 0.0001	< 0.05
W	≤ 0.0001	< 0.01
Sp	≤ 0.0001	**ns**
S x W	≤ 0.0001	**ns**
S x Sp	≤ 0.0001	**ns**
W X Sp	≤ 0.0001	**ns**
L x S	≤ 0.0001	≤ 0.0001
L x W	≤ 0.0001	≤ 0.0001
L x Sp	≤ 0.0001	≤ 0.0001
L x S x W	≤ 0.0001	≤ 0.0001
L x S x Sp	≤ 0.0001	≤ 0.0001
L x W x Sp	**ns**	**ns**
S x W x Sp	**ns**	**ns**
L x S x W x Sp	**ns**	**ns**

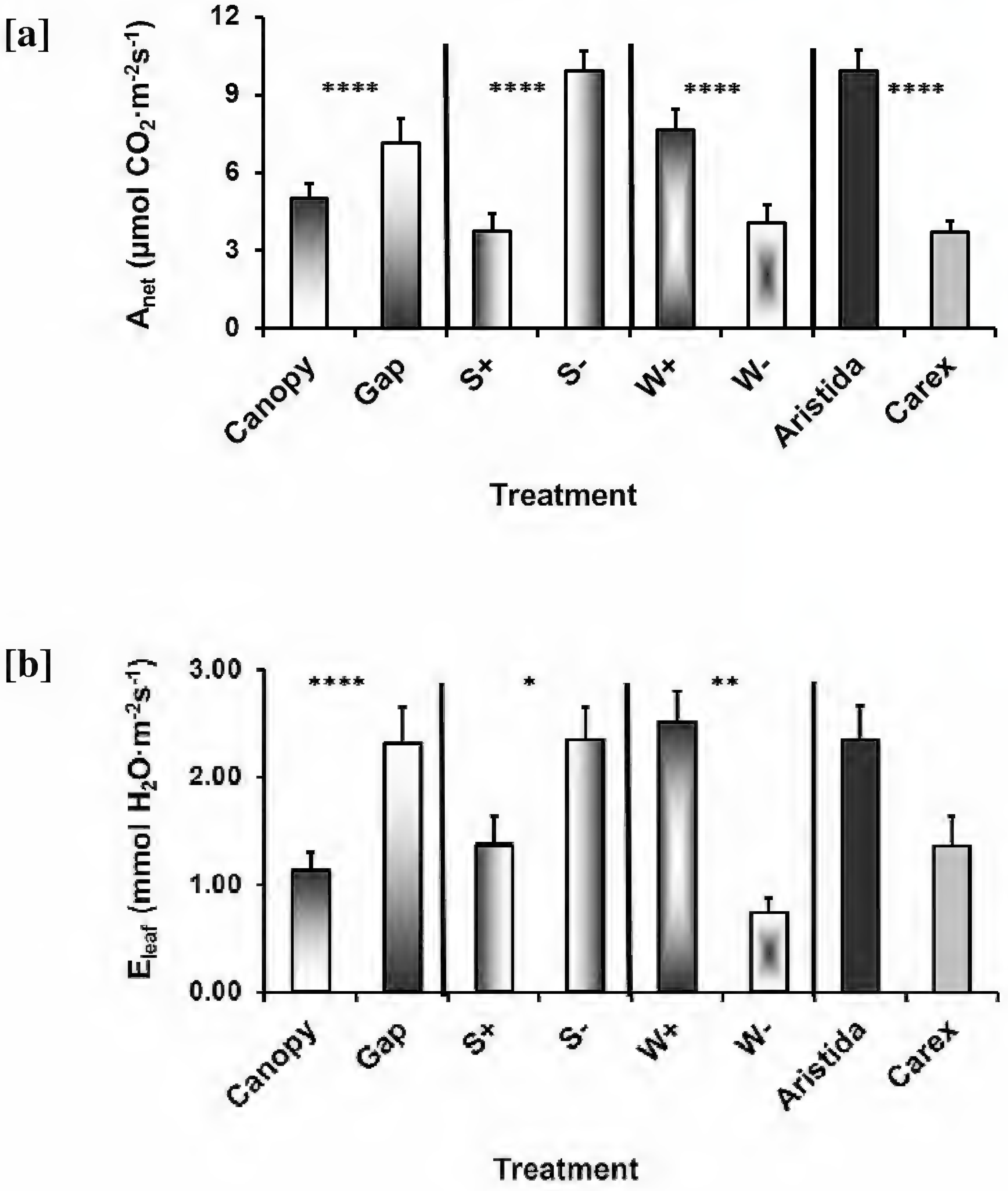

Figure 1. Mean main effect plots (x± *SE*) for [a, upper]-net photosynthesis (A_{net}) and [b, lower]-transpiration (E_{leaf}) in August. Treatments tested were for the effects of location (Canopy or Gap), shade (S+ or S-), water (W+ or W-) and species (*Aristida* or *Carex*). Vertical bars separate treatment groups. Treatment groups with symbols above indicate significant main effects. * = $P < 0.05$, ** = $P < 0.01$ and **** = $P \leq 0.0001$.

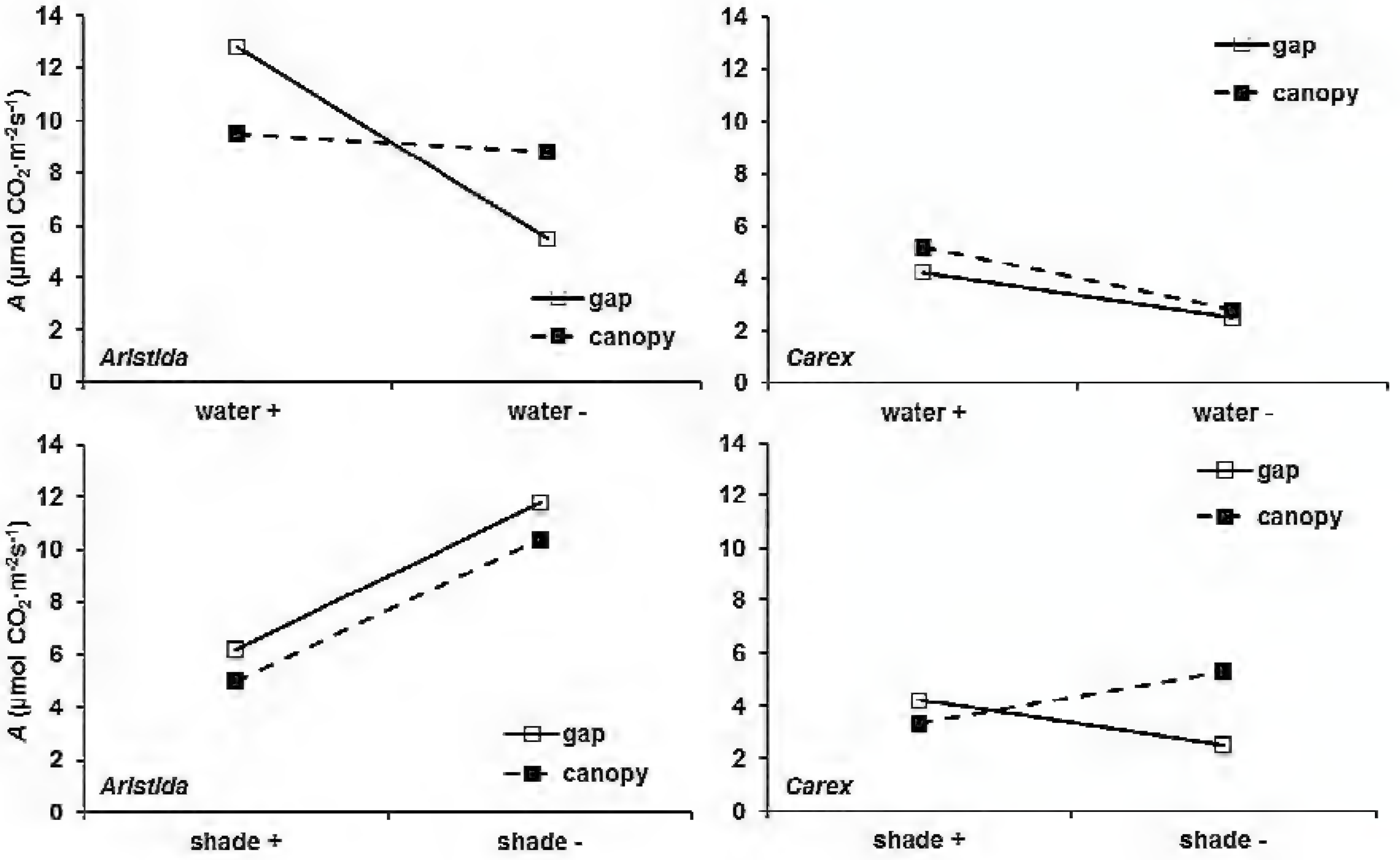

Figure 2. Means plots of the shade *x* water interaction on net photosynthesis (A_{net}) for *Aristida longiseta* (left side) and *Carex planostachys* (right side) in August. Measurements presented are treatment average on plants occurring below the *J. ashei* canopy and in an associated intercanopy gap. Treatments were supplemental water and no added water (W+ and W-) upper plots and supplemental shade and no added shade (S+ and S-) lower plots.

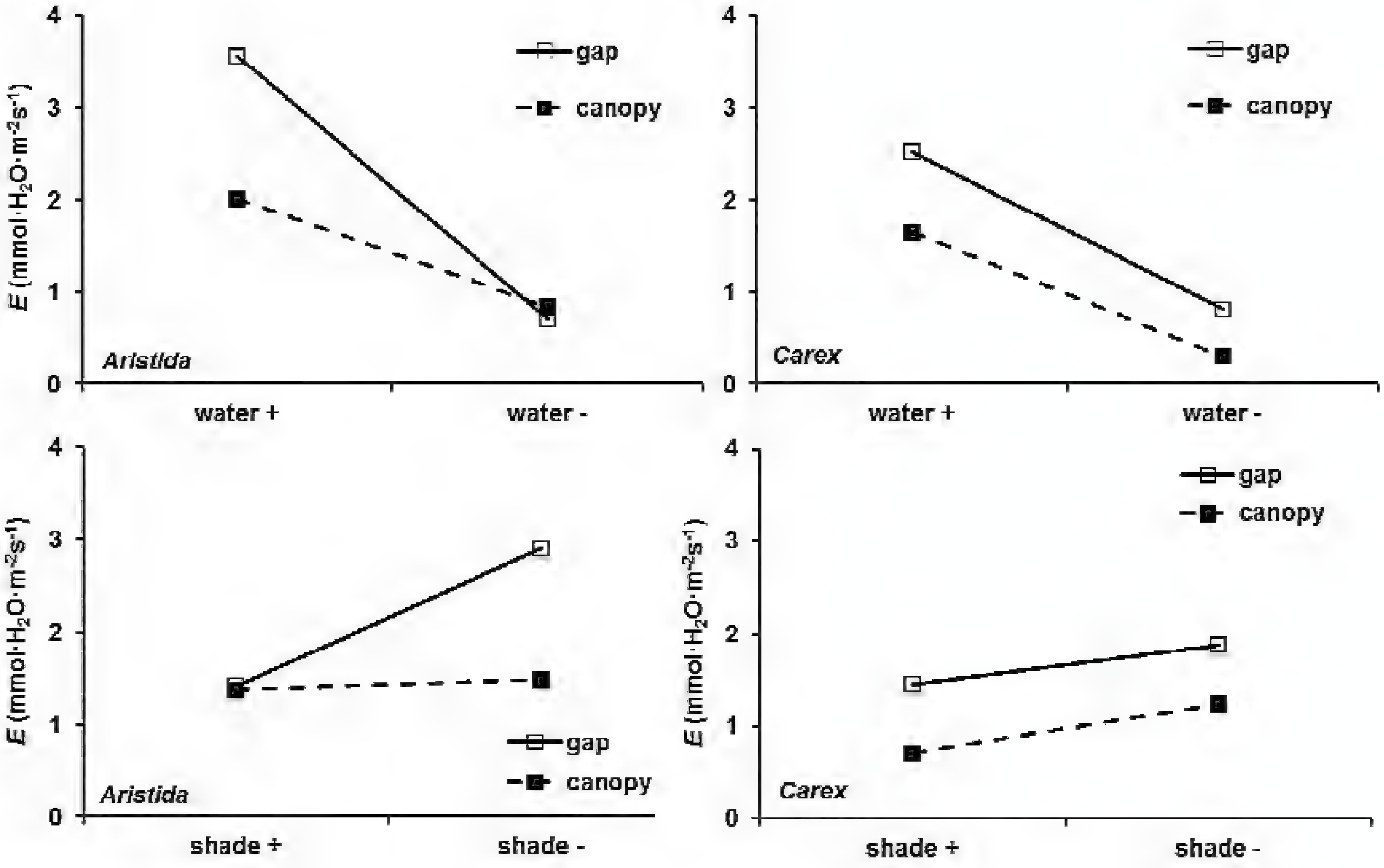

Figure 3. Means plots of the shade *x* water interaction on leaf transpiration (E_{leaf}) for *Aristia longiseta* and *Carex planostachys* in August. Measurements presented are treatment average on plants occurring below the *J. ashei* canopy and in an associated intercanopy gap. Treatments were supplemental water and no added water (W+ and W-) upper plots and supplemental shade and no added shade (S+ and S-) lower plots.

Phylogenetic placement of *Blastocladiopsis parva* (*Blastocladiomycota*)

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ABSTRACT

Blastocladiopsis parva was originally placed in *Blastocladia* under an invalid name, and was later segregated out of that genus based on resting spore morphology. Later authors showed resting spore morphology was not sufficient to delineate a new genus and advocated using physiology and growth form but did not formally amend the generic description. Using an isolate obtained in Alabama and amplicon based next generation sequencing, we place *Blastocladiopsis parva* in a molecular phylogeny, which confirms separating it from *Blastocladia* and emend the generic description to include growth form and physiology.Published on-line www.phytologia.org *Phytologia 100(1): 104-110 (Mar 16, 2018).* ISSN 030319430.

KEY WORDS: Ion Torrent, taxonomy, zoosporic fungi

Blastocladiomycota are an ecologically diverse zoosporic fungal lineage (Porter et al. 2011). A recent molecular phylogeny of the *Blastocladiomycota* revealed the non-monophyly of the family *Blastocladiaceae*, which contains the saprobic genera *Allomyces*, *Microallomyces*, *Blastocladia*, *Blastocladiella*, and *Blastocladiopsis* (Porter at el 2011). In the phylogeny of Porter et al. (2011), representatives of *Allomyces*, *Microallomyces*, and *Blastocladia* form a well-supported clade corresponding to the *Blastocladiaceae* while representatives of *Blastocladiella* group with representatives of *Catenariaceae* (Porter et al. 2011). Neither *Blastocladiopsis parva* (Whiffen) Sparrow, the type of *Blastocladiopsis*, or *Blastocladiopsis elegans* J. A. Robertson were included in the analysis (Porter et al. 2011), and their phylogenetic placement remains unknown.

Blastocladiopsis parva was originally described by Whiffen (1943) under the invalid name "*Blastocladia parva*". Whiffen failed to provide the required Latin description necessary in 1943 (McNeill et al. 2012), hence the binomial was invalidly published. The thallus of her fungus consisted of nonspetate, sub-dichotomously to dichotomously branched filaments with apical zoosporangia or resting spores (Whiffen 1943). It is saprobic in the soil and grows on snake skin and grass baits (Sparrow 1960). Sparrow (1950) reclassified Whiffen's fungus by placing it into the newly described genus *Blastocladiopsis* based on the resting spores being loose in the sporangia and having smooth walls, which contrasted with other members of *Blastocladia*. Sparrow (1950) provided a Latin description of the genus and included only one species, *B. parva*. By doing so, Sparrow validated both the generic name and the species binomial via a "description generic-specifica" as allowed by Art. 38.5 and as defined by Art. 38.6 (McNeill et al. 2012). Emerson and Robertson (1974) found resting spore characters to be insufficient to delineate genera within the *Blastocladiaceae* and suggested that generic boundaries be reformulated using physiology and growth form. They felt better features to distinguish *Blastocladiopsis* from *Blastocladia* were its growth on protein rich substrates (e.g., keratin) and the fact that "hyphal tips were not renewed by sympodial branching", i.e., growth along each branch was "strictly determinant" (Emerson and Robertson 1974). During a recent survey of chytrid diversity, we observed *Blastocladiopsis parva* in agricultural soils baited with snake skin, and sequenced the LSU rDNA region using next-generation sequencing. Herein, we report *Blastocladiopsis parva*'s placement within the phylogeny of Porter et al.

(2011) and emend the description of *Blastocladiopsis* to include Emerson and Robertson's (1974) suggestions.

MATERIALS AND METHODS

We collected soil from a ditch beside an agricultural field off Hwy 25 in Faunsdale, Alabama, USA (32.367241, −87.627883) in a sterile whirl-top bag and transported it on ice. In the laboratory, we placed a portion of the soil in a sterile Petri dish with sterile water and baited it with sterile snake skin. We photographed thalli of *Blastocladiopsis parva* using a Zeiss Axioskop with a Zeiss AxioCam MRc3 camera.

DNA extractions

We placed snake skin with month-old thalli of *Blastocladiopsis parva* in two sterile 2 mL tubes. To each tube, we added 200 µL of PL1 solution from a NucleoSpin Plant II DNA extraction kit (Macherey-Nagel Inc., Bethlehem, PA) and glass beads. We ground the samples with a mortar for 8 mins, added 10µl of RNase A and 10 µL Proteinase K and vortexed for 1 min. We then incubated the samples at 65C for 30 mins with brief (10s) vortexing every 15 mins. We transferred the samples to a 4C ice bath for 36 mins, and then incubated them for 5 mins at 65C. To each sample, we added 100 µL of chloroform and vortexed for 1 min. We then centrifuged the samples for 15 mins at 13k rpms. We transferred the aqueous layer to a sterile 2mL and brought the volume of the samples to the 0.75 mL line with sterile water. We then added 750 µL of phenol:chloroform to each samples. Samples were hand-shook vigorously for 3 mins and centrifuged for 5 mins at 13k rpms. The aqueous layer was transferred to a sterile 2mL tube and combined with 1mL of chloroform. The samples were hand-shook vigorously for 3mins and then centrifuged for 5 mins at 13k rpms. The aqueous layer was transferred to a new 2mL tube. We added 100 µL NaOAc and 1mL of isopropanol to each sample. Samples were incubated at −20C for 30mins before centrifuging for 15mins at 13k rpms. We discarded the supernant and added 500 µL of cold 75% ethanol. Samples were vortexed for 10 s and centrifuged for 3 mins at 13k rpms. The supernant was discarded and a second wash with 500 µL of cold 75% ethanol was performed. The supernant was discarded, and the tubes were dried under a laminar flow for 30 mins. The DNA pellet was re-suspended in 100 µL of TE buffer.

Amplicon generation & sequencing

DNA was amplified using fusion barcoded LROR (5'-ACCCGCTGAACTTAAGC-3' (Vilgalys and Hester 1990) and EDF360r (5'-TACTTGTICGCTATCGGTCTC-3'), a fungal affinity reverse primer targeting 300bp of the LSU rDNA region (Picard 2017). PCR amplifications were performed on 50ng of DNA under the following conditions: an initial denaturing step of 2 min at 94°C; 30 cycles of 1 min 94°C, 30 s 48°C, 15 s 51°C, and 1 min 72°C; and a final elongation step of 7 min at 72°C. Four replicate amplifications were pooled and dried in a 37°C incubator. The pellet was resuspended using 25µL of sterile water. Amplicons were gel purified via a NucleoSpin Extraction II kit (#740609.250; Macherey-Nagel, Inc.), quantified by fluorometry using a Qbit 2.0 (Invitrogen), and sent to Duke University for ion semiconductor (Ion Torrent) sequencing on a 314-chip using 400bp read chemistry (Sequencing and Genomic Technologies Shared Resource, Duke University, Durham, NC).

Analysis of next-generation amplicon data

The sample was run on the same chip as the samples in Davis et al. (in revision); thus, the initial processing was the same. Sequences were filtered based on a minimum average Phred quality score of 25 based on a sliding window of 50 bases, a size requirement of 200–350 bp, homopolymer run less than 6 bp, and a primer mismatch of 1 bp in QIIME 1.8.0 (Caporaso et al. 2010). Chimeras were detected with USEARCH v.6.1.544 (Edgar 2010) and the Ribosomal Database Project's 28S fungal database (No. 11; Liu et al. 2012) and removed in QIIME. OTUs were grouped at a 95% similarity threshold using uclust (Edgar 2010) in QIIME, and a representative set of sequences was generated using the most abundant sequence belonging to an OTU. An OTU table was generated and filtered to include only the *Blastocaldiopsis parva* sample. This OTU table was used to pull the appropriate sequences from the representative sequences. Taxonomy was assigned using the Ribosomal Database Project's naïve-bayesian classifier 2.7 (RDB-NBC; Liu et al. 2012), which was retrained using a version of the RDP-NBC 28S fungal database No.11 that was hand curated to update the taxonomy of the chytrid sequences and to add additional chytrid sequences deposited in GenBank from taxonomic revisions and species descriptions. OTUs classified as Fungi at >75% support and Blastocladiomycota at >65% support were selected for phylogenetic analysis. Raw sequences are submitted to the NCBI Sequence Read Archive (PRJNA395910). Sequences representing the OTUs were submitted to GenBank (OTU 1284: MG925664; OTU 2114: MG925663). All scripts are deposited on GitHub (https://github.com/wjdavis90/Blastocladioposis_parva).

Phylogenetic analysis

The complete alignment of Porter et al. (2011) was downloaded from Treebase (treebase.org). The OTU sequences were added to the alignment using the 'add fragments' function of mafft v. 7.294 (Katoh et al. 2002; Katoh and Frith 2012; Katoh and Standley 2013). The alignment was filtered using Gblocks v. 0.91b (Castresana 200) with the minimum number of sequences for conserved and flanking positions set to 38, the maximum number of contiguous nonconserved positions set to 8, the minimum length of a block set to 5, and gaps allowed. Maximum likelihood (ML) trees were inferred using the parallel version of RAxML 8 (Stamatakis 2014). Trees were inferred using the GTR + GAMMA model of sequence evolution and bootstrapped with 1000 replicates.

RESULTS

The morphology of our isolate matched the description given by Whiffen (1943) and Sparrow (1950). Thalli exhibited determinant, dichotomously branching growth with terminal resting spores (Fig. 1A). Resting spores were detached from the sporangial wall and had smooth walls (Fig. 1B). Zoosporangia were not observed.

There were 18590 sequences to start. After quality control, there were 5893 sequences that grouped into 78 OTUs. Fungi was the most abundant taxon (66%) followed by Stramenopiles (26%) and Rhizaria (3%). Only OTUS 1284 and 2114 from the sample were identified as members of *Blastocladiomycota*.

There were 15689 positions in the original alignment, and Gblocks reduced it to 2946 positions (18%). The inferred tree had low bootstrap support (<50%) at multiple nodes, especially compared to the tree presented in Porter et al. (2011). Thus, we inferred a new phylogeny using all of the sites. The phylogeny inferred from the Gblocks selected positions is available on GitHub. In the phylogeny inferred from the full alignment, OTUs 1284 and 2114 are sister to a clade containing *Blastocladiella britainnica*, *Blastocladiella* sp. JEL363, *Blastocladiella* sp. JEL440 and strains of *Catenaria* with 85% bootstrap support (Fig. 2). *Blastocladia pringsheimii*, the type of the genus, forms a well-supported clade (88% bootstrap support) with representatives of *Allomyces* and *Microallomyces* in the *Blastocladiaceae* (Fig. 2).

TAXONOMY

Blastocladiopsis Sparrow, J. Wash. Acad. Sci. 40: 52. 1950 emend. W. J. Davis [this publication]
MycoBank 20080

Typification: *Blastocladiopsis parva* Sparrow

Etymology: *Blastocladi-* referring to the genus *Blastocladia*; *-opsis* from the Greek *opsis*= appearance.

Description: THALLUS on protein rich substrates filamentous, sub-dichotomously to dichotomously branched. RHIZOIDS course and branched. GROWTH determinant, sympodial branching after development of sporangia absent. ZOOSPORANGIA infrequent, irregular to cylindrical, borne singly with one to several discharge papillae. RESTING SPORES borne singly, held loosely within sporangia, gold, amber or dark brown with smooth to reticulate think walls and one to several discharge pores or papillae upon germination. ZOOSPORES posteriorly uniflagellate with several lipid globules and a nuclear cap. Differs from *Blastocladia* by growing as an obligate aerobe. Differs from *Blastocladia* and *Blastocladiella* by branching subdichotomously to dichotomously. Differs from *Allomyces* by showing strictly determinant growth; no sympodial branching after the formation of sporangia.

Blastocladiopsis parva Sparrow, J. Wash. Acad. Sci. 40: 52. 1950
MycoBank 293721
"*Blastocladia parva*" Whiffen, J. Elisha Mitchell Sci. Soc. 59: 40. 1943 [nom invalid.]

Typification: Soil from Terrell, Texas, USA. (**Holotype**-Fig. 17, Whiffen J. Elisha Mitchell Sci. Soc. 1943)

Etymology: from the Latin *parvus* = small, little

Description: English description provided under the name "*Blastocladia parva*" Whiffen [in 1943 a nom. invalid., Melbourne Code Art. 39.1]

THALLUS, as described by Whiffen (1943) and again by Sparrow (1950), 300µm or more in length, 12–50µm in diameter, subdichotomously to dichotomously branched. ZOOSPORANGIA infrequent, irregular to cylindrical with one to six discharge papillae. RESTING SPORES spherical to ellipsoid, gold to amber-colored, smooth-walled, lying loosely in sporangium with one to two discharge pores upon germination. ZOOSPORES posteriorly uniflagellate. Differs from *Blastocladiopsis elegans* by the smooth resting sporangia walls.

Additional specimens examined: USA, ALABAMA: Faunsdale, Hwy25, roadside ditch beside an agricultural field. Snake skin, soil. GenBank MG925664 (28s, OTU 1284) and MG925663 (28s, OTU 2114).

DISCUSSION

Sparrow (1950) described *Blastocladiopsis* distinguishing it from members of *Blastocladia* based on the space separating the thick wall of the resting spore from the thin wall of the sporangium and the smooth nature of the resting spore walls. Emerson and Robertson (1974) reviewed several species of the *Blastocladiaceae* and found these to be a common character states in the family. They proposed that the genera be delineated based on physiology and growth form (Emerson and Robertson 1974). They suggested the main character state separating *Blastocladiopsis* from *Blastocladia* was obligate aerobic growth on protein-rich substrates, and the main character state separating it from *Blastocladiella* was its branched growth form (Emerson and Robertson 1974). They concluded the fundamental character state defining this genus was that "the branches of *Blastocladiopsis* do not have the capacity for renewed growth by sympodial branching once they have formed sporangia at their apices" (Emerson and Robertson 1974, p. 314). Following Emerson and Robertson's (1974) concept of the genus, Robertson (1976) described *Blastocladiopsis elegans* even though the species had reticulate resting spores that essentially filled the sporangia. Thus, we emend Sparrow's (1950) description to incorporate and emphasize the physiology and growth form of this genus and de-emphasize the formation and structure of the resting spores following Emerson and Robertson (1974) and Robertson (1976). Our molecular phylogeny places *B. parva* in a clade distinct from the type of *Blastocladia*, which validates Sparrow's erection of a new genus for it. In our molecular phylogeny, *Blastocladiopsis parva* is sister to strains representing *Blastocladiella*. Since *Blastocladiopsis* differs morphologically from *Blastocladiella* by its branched growth form (Emerson and Robertson 1974), *Blastocladiella* is currently non-monophyletic, and neither the type species of *Blastocladiella* or *Blastocladiopsis elegans* are represented in the current molecular phylogeny (Porter et al. 2011), we feel it is appropriate to retain *Blastocladiopsis* as its own genus until the placement of *Blastocladiella simplex* and *Blastocladiopsis elegans* is known.

ACKNOWLEDGMENTS

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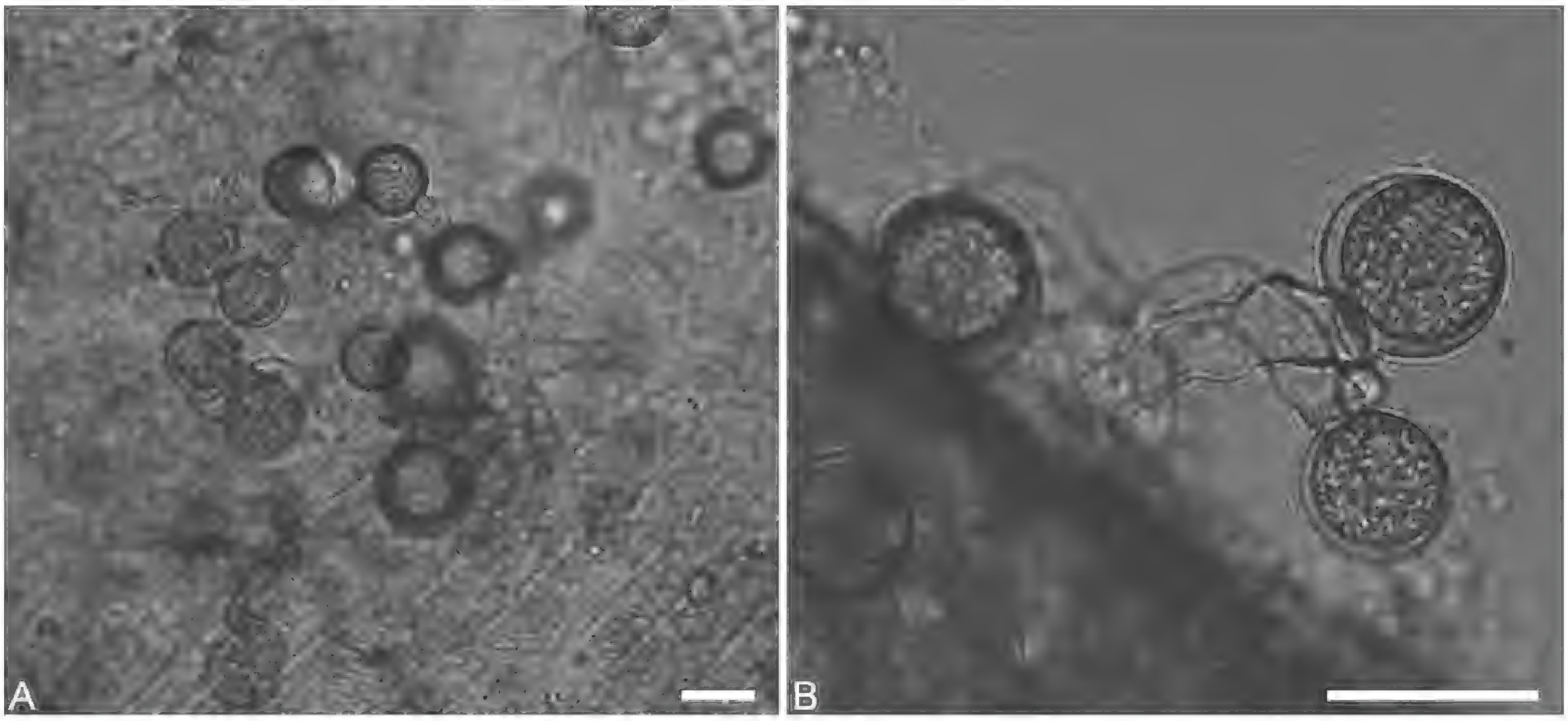

Figure 1. Light micrographs of *Blastocladopsis parva*. A. Dichotomously branched thallus with multiple resting spores. Scale bar = 30µm. B. Close up of resting spores showing the smooth wall and detachment from the sporangium wall. Scale bar =30µm.

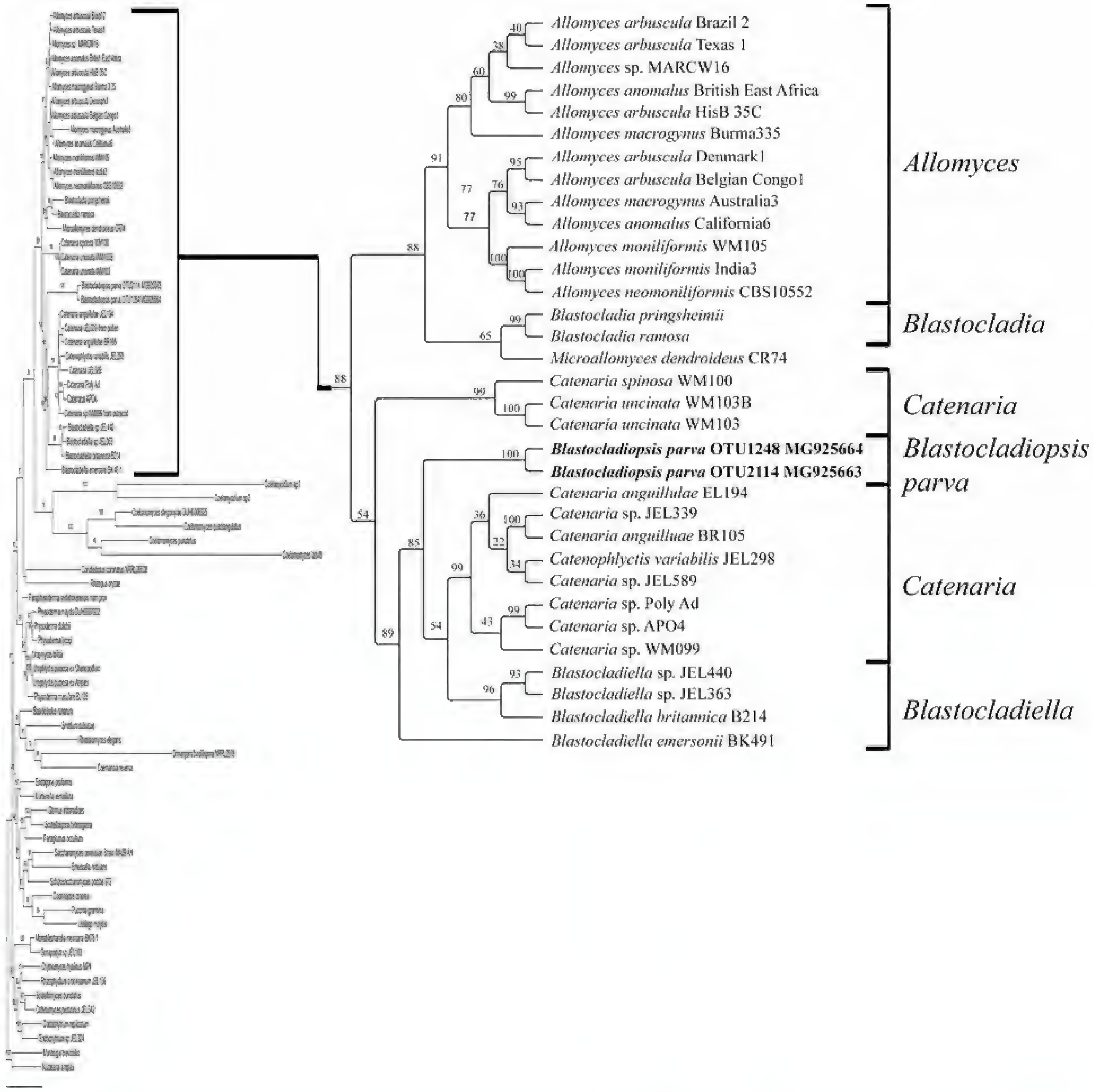
Allomyces arbuscula Brazil 2
Allomyces arbuscula Texas 1
Allomyces sp. MARCW16
Allomyces anomalus British East Africa
Allomyces arbuscula HisB 35C
Allomyces macrogynus Burma335
Allomyces arbuscula Denmark1
Allomyces arbuscula Belgian Congo1
Allomyces macrogynus Australia3
Allomyces anomalus California6
Allomyces moniliformis WM105
Allomyces moniliformis India3
Allomyces neomoniliformis CBS10552
Blastocladia pringsheimii
Blastocladia ramosa
Microallomyces dendroideus CR74
Catenaria spinosa WM100
Catenaria uncinata WM103B
Catenaria uncinata WM103
Blastocladiopsis parva OTU1248 MG925664
Blastocladiopsis parva OTU2114 MG925663
Catenaria anguillulae EL194
Catenaria sp. JEL339
Catenaria anguilluae BR105
Catenophlyctis variabilis JEL298
Catenaria sp. JEL589
Catenaria sp. Poly Ad
Catenaria sp. APO4
Catenaria sp. WM099
Blastocladiella sp. JEL440
Blastocladiella sp. JEL363
Blastocladiella britannica B214
Blastocladiella emersonii BK491
Allomyces
Blastocladia
Catenaria
Blastocladiopsis parva
Catenaria
Blastocladiella
40
38
60
99
80
91
95
76
77
93
77
100
100
88
99
65
88
99
100
100
54
36
100
22
34
85
99
99
43
54
89
93
96

Figure 2. Inferred maximum likelihood phylogeny for *Blastocladiomycota*. The analysis included 71 sequences and 15689 positions. The full phylogeny is shown on the left and is rooted with *Monosiga brevicolis* and *Nuclearia simplex*; on the right, the *Blastocladiaceae* and *Catenariaceae* are shown as a cladogram. Bar = 1.0 changes per site.

Seed cone volume, a new methodology for a metric to differentiate *Hesperocyparis* (western cypress, Cupressaceae) taxa

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ABSTRACT

Treating the irregularly surfaced seed cone of Monterey cypress (*Hesperocyparis macrocarpa*) as an ellipsoid, cone volume (cm^3) for 25 sampled cones was calculated by using cone length and diameter measurements in the mathematical volume formula for a spheroid. A graduated cylinder was used to directly measure volume (ml) of the same 25 cones to compare the two volume methodologies. The two methods were always within 0.9 cm^3 (ml) of each other for each cone sampled and were not statistically different. The easily measured seed cone length and width of the calculated methodology may make for precise cone volume comparison among populations and taxa. Published on-line www.phytologia.org *Phytologia 100(1): 111-116 (Mar 16, 2018).* ISSN 030319430.

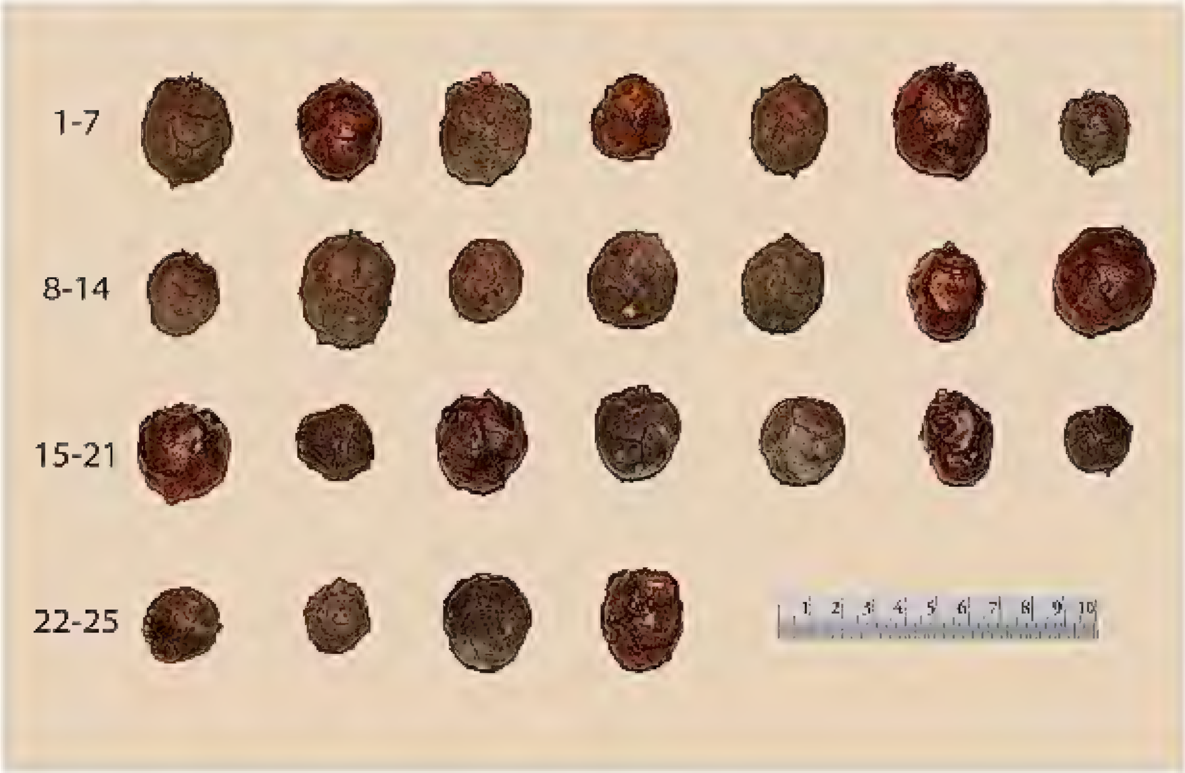
KEY WORDS: *Hesperocyparis*, *H. abramsiana*, *H. macrocarpa, Cupressus,* western cypress, New World cypress, seed cone, seed cone volume, seed cone density, taxonomy, Cupressaceae.

Seed cone (ovulate cone or megastrobilus) length is a measurable character or metric that has been used in taxonomic keys to distinguish among pine family (Pinaceae) genera (*Picea*, *Pseudotsuga*, and *Tsuga*) and various California pines (*Pinus*) (Baldwin et al. 2012). This metric was similarly used in the cypress family (Cupressaceae), along with leaf morphology, to distinguish between the coast redwood (*Sequoia*) and giant sequoia (*Sequoiadendron*) (Bartel 2012). Additionally seed cone length and other cone characteristics (e.g., shape, color, woody versus fleshy) were used in part to distinguish among other cypress family genera (*Callitropsis*, *Chamaecyparis*, *Juniperus*, *Hesperocyparis*) and between western or New World cypress (*Hesperocyparis*) species in California (Bartel 2012). In one case, ranges in seed cone length and diameter were used in tandem to depict obvious differences in overall cone size to differentiate between two varieties of *H. abramsiana* (Bartel 2012).

Maturing the second year, the woody seed cones of western cypress (*Hesperocyparis*) are serotinous and, thus, generally remain closed and attached beyond maturity (> 2 years) (Bartel 2012). Cones range from 10-50 mm in length and are generally spheric to widely cylindric in shape (Bartel 2012). Pairs of decussate cone scales are peltately attached around the cone axis, which is often longer than the cone width or diameter. Because the range of seed cone length and width measurements typically overlap between taxa and populations, comparing cone volumes may make seed cone size differences more readily apparent than by length and width measurements alone. This paper presents a new methodology for a metric to calculate seed cone volume and discusses its possible use.

MATERIALS AND METHODS

Twenty-five (25) mature seed cones were collected from 10 naturalized Monterey cypress (*Hesperocyparis macrocarpa*) trees growing within the highway divider of Carlsbad Boulevard near its intersection with Avenida Encinas in Carlsbad, CA. A variety of sizes and shapes, from spheric to widely cylindric, were included in the 25 sampled cones (Fig. 1). The seed cones were measured using a DoWorld Tools 6-inch electronic digital caliper at a resolution of 0.01 mm. Cone length was measured from stem to cone bottom, while two cone diameter or width readings were made equidistant along the cone axis at 90° from each other. The seed volume in cubic centimeters (cm^3) was calculated in Microsoft Excel using the volume formula for an ellipsoid (Burnside 2017) [$V = (4/3)\cdot\pi\cdot a\cdot b\cdot c$] where a = cone length radius, b = first cone diameter radius, and c = second cone diameter radius (Fig 2).



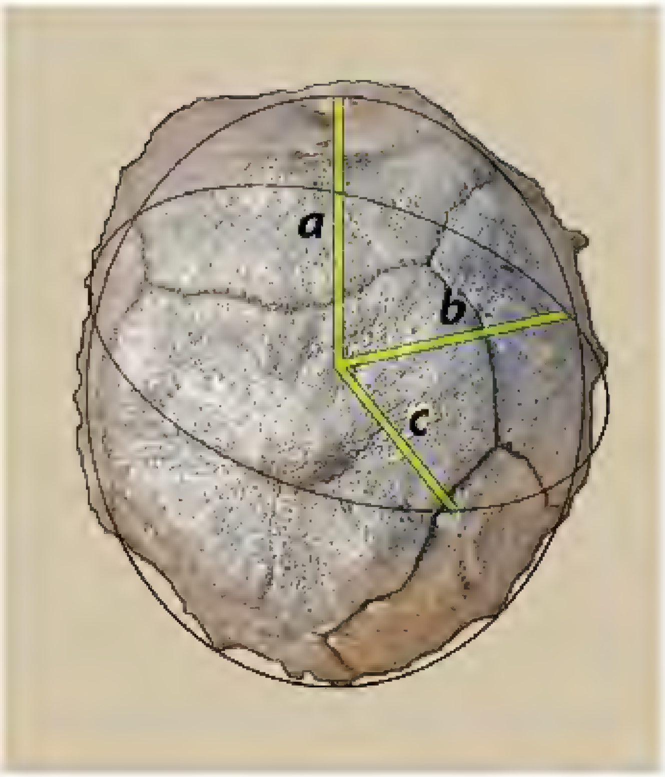


Figure 1. The 25 sampled seed cones identified by cone number. Figure 2. Seed cone depicting the three measured radii within a cone used to calculate volume. All angles between each radius pair are 90°.

After the seed cones were measured with the caliper, the cones were placed one at a time into a 250 ml graduated cylinder with a measured volume of water. The measured volume in milliliters (ml) for each sampled cone was the difference to the nearest 0.5 ml between the cylinder readings with and without the seed cone.

RESULTS AND DISCUSSION

The cylinder measured and caliper calculated seed cone volumes and the differences between these methods are given for each of the 25 sampled seed cones in Table 1. While the two methods produced identical volume numbers only once, the two methods always were within 0.9 cm^3 (ml) of each other. In Excel, the two methods were analyzed using a t-Test of paired two sample for means, which is presented in Table 2. This statistical test is used to determine whether the null hypothesis (H_0) should be rejected that the difference between the means of the two methods is zero ($\mu_d = 0$). At a 95% confidence level ($\alpha = 0.05$), the t-Statistic number is smaller than the t Critical two-tail number. Consequently, the null hypothesis is not rejected and, therefore, the means from the two volume measuring methods are not statistically different.

In determining which method to choose to measure the volume of an irregularly shaped object, Bruce and Schumacher (1950) noted that measuring the displaced volume was the most accurate method. However in practice the cylinder methodology used in this study for seed cones had issues because it requires two readings (one with and one without the cone) that are approximated to the nearest 0.5 ml, which impacts accuracy. In addition, cones occasionally floated in the cylinder water making readings

Table 1. Cylinder measured and caliper calculated seed cone volumes and the differences between these methods for each of the 25 sampled seed cones

Cone number	Cylinder measured volume (ml)	Caliper calculated volume (cm^3)	Difference of calculated volume to measured volume
1	12.0	11.8	-0.2
2	10.5	10.6	0.1
3	14.0	14.0	0.0
4	7.5	8.0	0.5
5	9.5	9.2	-0.3
6	16.0	15.5	-0.5
7	6.0	6.2	0.2
8	7.0	6.8	-0.2
9	15.5	14.9	-0.6
10	6.0	6.5	0.5
11	11.5	11.9	0.4
12	9.5	9.0	-0.5
13	8.0	7.6	-0.4
14	16.5	16.7	0.2
15	12.5	11.6	-0.9
16	7.0	6.3	-0.7
17	12.0	11.8	-0.2
18	9.5	10.3	0.8
19	9.5	9.7	0.2
20	7.5	6.6	-0.9
21	4.5	4.2	-0.3
22	5.0	5.3	0.3
23	4.5	4.3	-0.2
24	10.5	10.3	-0.2
25	11.0	10.3	-0.7

Table 2. Results of t-Test of paired two sample for means for the cylinder measured and caliper calculated seed cone volumes

	Cylinder measured volume (ml)	Caliper calculated volume (cm3)
Mean	9.72	9.58
Variance	12.231	11.911
Observations	25	25
Pearson Correlation Coefficient	0.991	
Hypothesized Mean Difference	0	
Degrees of Freedom	24	
t Statistic	1.579	
P(T<=t) two-tail	0.127	
t Critical two-tail	2.064	

difficult. Moreover, because the cylinder methodology requires that the cone be immersed in water, seed cones likely would require drying so as to not affect their further use in the study. Lastly, caliper measurement of seed cones is already occurring, along with other observations like number of cone scale pairs per cone. As a result, using the caliper method to precisely obtain seed cone volume would only come at the cost of further analyzing previously collected data in a spreadsheet.

Adams and Bartel (2009) used combined Inter-Simple Sequence Repeats (ISSRs) and terpenoid data to support the varietal recognition of the Butano Ridge population of *Hesperocyparis abramsiana* from the other four populations. To distinguish *H. abramsiana* var. *butanoensis*, the authors used mean seed cone length and diameter in a table (Adams and Bartel 2009). In developing a taxonomic key, Bartel (2012) primarily used ranges of seed cone length and diameter in tandem to separate the varieties of *H. abramsiana*. Using the original caliper data of seed cone length and diameter from Adams and Bartel (2009) to calculate cone volumes and inserting those values into an updated table from the same article, the differences in mean cone volume between the two varieties of *Hesperocyparis abramsiana* in Table 3 are more obvious. McMillan (1952) used a photograph of a "random selection" of seed cones from the then three known populations to demonstrate the "striking difference" in cone size. Recently in a self-admitted "too small cone sample size," Malone et al. (2012) provided additional seed cone images and a confusing table of the five populations of *H. abramsiana* with 3 "indices" involving unexplained computations of seed cone length and/or width. Whereas using the mean cone volumes in addition to various cone length and width measurements in Table 3, the seed cones from Butano Ridge proved to be demonstrably larger ranging from nearly twice to more than four times the size of the other populations of *H. abramsiana*.

Table 3. Revised grove-by-grove comparison of *Hesperocyparis abramsiana* seed cones by adding mean cone volume data to Table 1 from Adams and Bartel (2009) and by adding cone length and width range data from Bartel (2012).

	Butano Ridge	**Eagle Rock**	**Bracken Brae**	**Bonny Doon**	**Majors Creek**
Mean of seed cone length (mm)	27.0	22.1	16.7	22.0	19.9
Range of seed cone length (mm)	22–32(35)	(14)16–25			
Mean of seed cone width (mm)	25.5	20.0	16.1	18.8	18.3
Range of seed cone diameter (mm)	22–31	14–22			
Mean number of scale pairs per cone	4.7	4.2	3.6	4.3	4.4
Mean of seed cone volume (cm^3)	9.2	4.1	1.9	4.2	4.8

While seed cone length and width are frequently used to identify differences in cypress (Wolf 1948, Farjon 2005, Bartel 2012), an attempt to quantify seed cone size or volume appears to be quite rare. Goggans and Posey (1968) measured "cone size" of several *Hesperocyparis* (*Cupressus*) taxa by weighing in grams 100 dried, open, and seed-free cones within each sampled population. The authors made general correlations by comparing the cone size of various populations in Arizona, California, New Mexico, and northern Mexico, including the Eagle Rock population of *H. abramsiana*. Ignoring that weighing cones after drying the cones and extracting the seeds, a 1 to 2 month process (Johnson and Karrfelt 2008), makes cone weight of no practicable use in the field, cone mass and cone volume are not synonymous given the varying densities of cones (see discussion below). Pandit and Ram (2002), working with *Cupressus torulosa* at two sites in the Himalayas of India, evidently measured seed cone weight of 100 seed cones per collection site prior to or during cone opening for this non-seritinous species. Though the authors reported that "cone size was the mean diameter of two axes (at right angle) of the cone measured" with a digital caliper, they also correctly noted that "cone density was the value of mass divided by the volume of the cone." Despite the confusing methodology section, the 2002 article focus was on the correlation of cone and seed moisture with seed germination and not taxonomy. Though not to calculate cone volume, Westman and Whittaker (1975) treated *H. pygmaea* cones as spheres to calculate cone surface area in a study of pygmy forest biomass in Mendocino County, California. Finally in a study of insect pests of *C. sempervirens* seed cones in Tunisia, Ben Jamaa and Roques (1999) used an unexplained formula to measure the volume of seed cones where l = cone length and w = cone width ($V = \pi \cdot l \cdot (3w^2 + l^2)/24$). Because a quick test using their formula for our *H. macrocarpa* seed cones resulted in larger cone volumes for 18 of the 25 cones and a statistically different mean volume from our calculated mean volume, we did not pursue the formula further.

Though compact digital scales make the accurate weighing of individual seed cones relatively easy in the field, cone weights likely will not correlate well with cone size because of the effects of cone maturation or aging on density. Kafton (1976) reported that while "full-size" seed cones of *Hesperocyparis macrocarpa* mature from greenish-brown (6 to 11 months in age) to brown (> 11 months in age) to grey in color (> 15 months), the cones continue to become "less soft and moist" as the cones age and desiccate. Older grey seed cones of *H. macrocarpa* often will naturally open up and release their seeds (Fig. 3). Despite collecting seed cones up to 32 years in age with viable seeds, Kuhlman (1986) in studying the Bracken Brae population of *Hesperocyparis abramsiana* reported that a slow release of seeds from trees appears to be a result of ongoing vascular constriction in the cone peduncle resulting in a loss of fluids allowing the cone scales to open. In a quick test to compare the relative densities of young versus older mature seed cones, the calculated volumes and measured mass (digital scale with 0.1 gram resolution) were taken for 15 light brown (young) and 15 dull brown to grey (older) seed cones. Using the density formula $D = m/v$, where density (D) is equal to mass (m) divided by volume (v), the mean mass per unit volume for young cones was 1.12 and for older cones 1.00. In light of the fact that the density of water is 1, not surprisingly 13 of the 15 young cones sank in water, while 12 of the 15 older cones floated in the water. Where species like *H. abramsiana* retain their closed cones for decades, this density difference likely will be greater for the oldest cones.

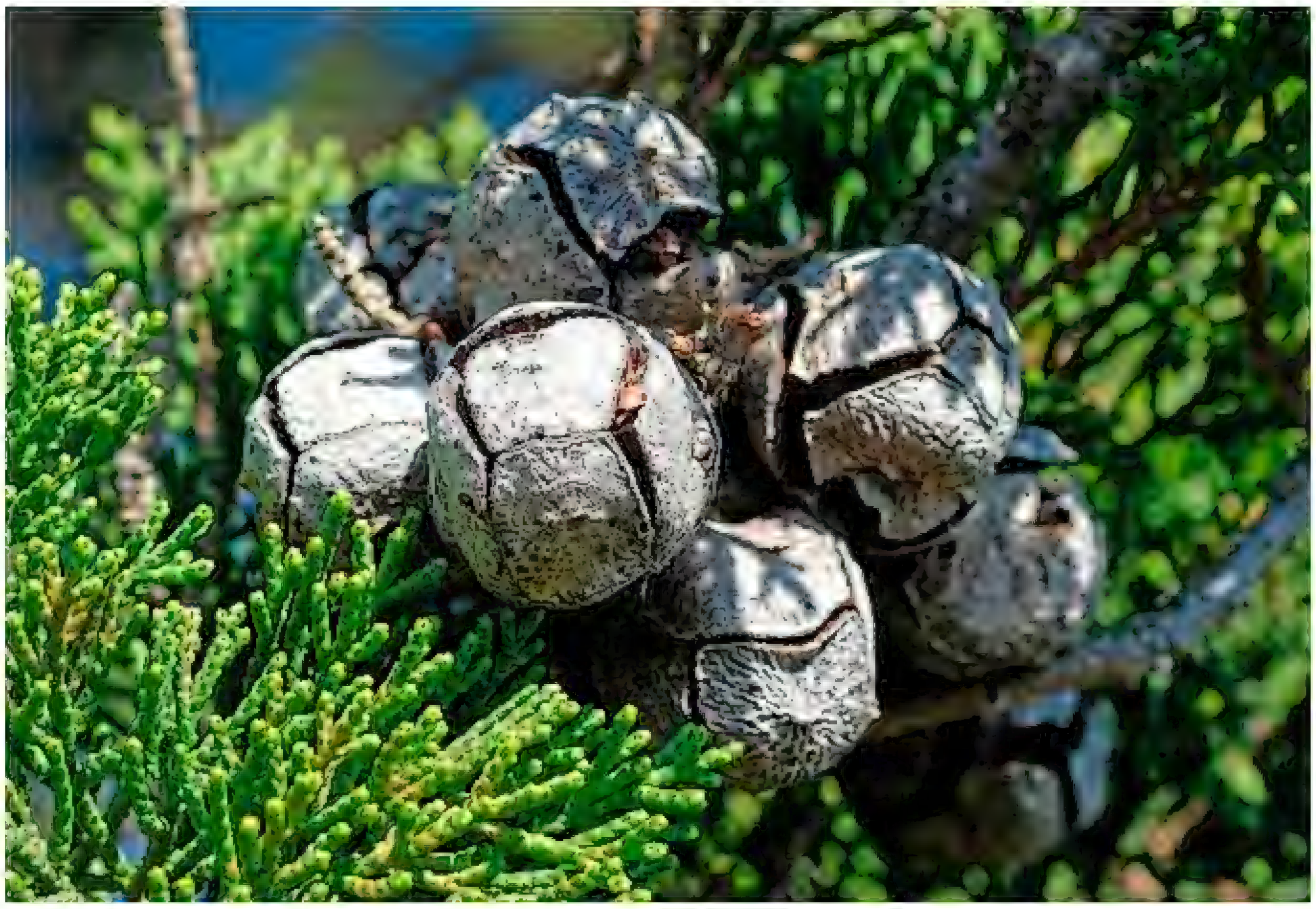


Figure 3. Naturally opening, older grey seed cones and dispersing seeds of *Hesperocyparis macrocarpa*.

CONCLUSIONS

Because seed cone volume is not an easily obtain metric in the field, seed cone length and diameter still will be required for taxonomic keys. Nonetheless, calculating seed cone volume appears to be a precise method to obtain an additional promising metric to easily recognize substantial differences in cone size. In the example of *Hesperocyparis abramsiana* var. *butanoensis* where using the mean cone volumes in addition to various cone length and width measurements in Table 3, the seed cones from Butano Ridge proved to be demonstrably larger using seed cone volume with cones ranging from nearly twice to more than four times the size of the other populations of *H. abramsiana*.

Though 3 radii were used in this article to calculate seed cone volume, typical cone measurements made in the field involve measuring 3 diameters that would then need to be halved. An alternative and simplified seed cone volume formula using diameters in lieu of radii would be $V = \pi \cdot a \cdot b \cdot c/6$ where a = cone length diameter, b = first cone width diameter reading made equidistant along the cone axis, and c = second cone width diameter reading at 90° from b.

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Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in Macedonia, Bosnia-Herzegovina, Croatia and Central and Southern Italy and relictual polymorphisms found in nrDNA

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ABSTRACT

Additional analyses of trnS-trnG and nrDNA from specimens from Bosnia-Herzegovina, southern and central, Italy, Croatia and Macedonia revealed the presence of *J. sabina* var. *balkanensis* in these areas west of the previously known populations in Greece, Bulgaria and western Turkey. Careful chromatogram analysis of eight (8) polymorphic sites in nrDNA revealed that nearly all of the populations of both var. *balkanensis* and var. *sabina* contained from 2 to 8 polymorphic sites. For these 8 heterozygous sites, two exclusive patterns were found in *J. sabina.* One type (GGACCCAG) was found in 16/62 plants and type 2 (ACGACAGT) was found in 4/62 plants. The majority of the plants examined (42/62) were heterozygous for 1 to 8 sites. These two nrDNA types appear to have arisen via hybridization with a *J. thurifera* ancestor. The two types appear in both v. *sabina* and v. *balkanensis* populations. Extant putative hybrids appear to have formed by crosses between present day type 1 and type 2 nrDNA. Published on-line www.phytologia.org *Phytologia 100(2): 117-127 (Jun 22, 2018).* ISSN 030319430.

KEY WORDS: *Juniperus sabina var. balkanensis, J. sabina*, distribution, nrDNA, trnS-trnG, chloroplast capture, ancient nrDNA heterozygotes.

Juniperus sabina var. *balkanensis* R. P. Adams & A. N. Tashev appears to be a product of hybrid origin between *J. sabina* L. and an ancestor of *J. thurifera* (Adams et al. 2016). Subsequent backcrossing to *J. sabina* (var. *sabina*) is hypothesized to have resulted in the capture of ancestral '*J. thurifera* like' chloroplast by *J. sabina* var. *balkanensis*. In *Juniperus*, Terry et al. (2000) suggested that chloroplast capture was involved in the distribution of cp haplotypes in *J. osteosperma* in western North America. More recently, Adams (2015a, b) found widespread hybridization and introgression between *J. maritima* and *J. scopulorum* in the Pacific northwest, with introgression from *J. maritima* into *J. scopulorum* eastward into Montana. The disparity between cpDNA and nuclear markers (nrDNA and maldehy) suggested that cp capture had occurred. In *Pinus* and other conifers, Hipkins et al. (1994) concluded that "past hybridization and associated 'chloroplast capture' can confuse the phylogenies of conifers.' Bouille et al. (2011) found significant topological differences in phylogenetic trees based on cpDNA (vs. mtDNA sequences) in *Picea* that suggested organelle capture. *Juniperus sabina* L. is a smooth leaf-margined, multi-seeded juniper of the eastern hemisphere. It is very widely distributed from Spain through Europe to Kazakhstan, western China, Mongolia and Siberia (Fig. 1, Adams 2014). *Juniperus sabina* has a range that is discontinuous between Europe and central Asia; the species is generally a shrub less than 1 m tall and ranges up to 1-2 m wide. However, in the Sierra Nevada of Spain, *J. sabina* is a horizontal shrub.

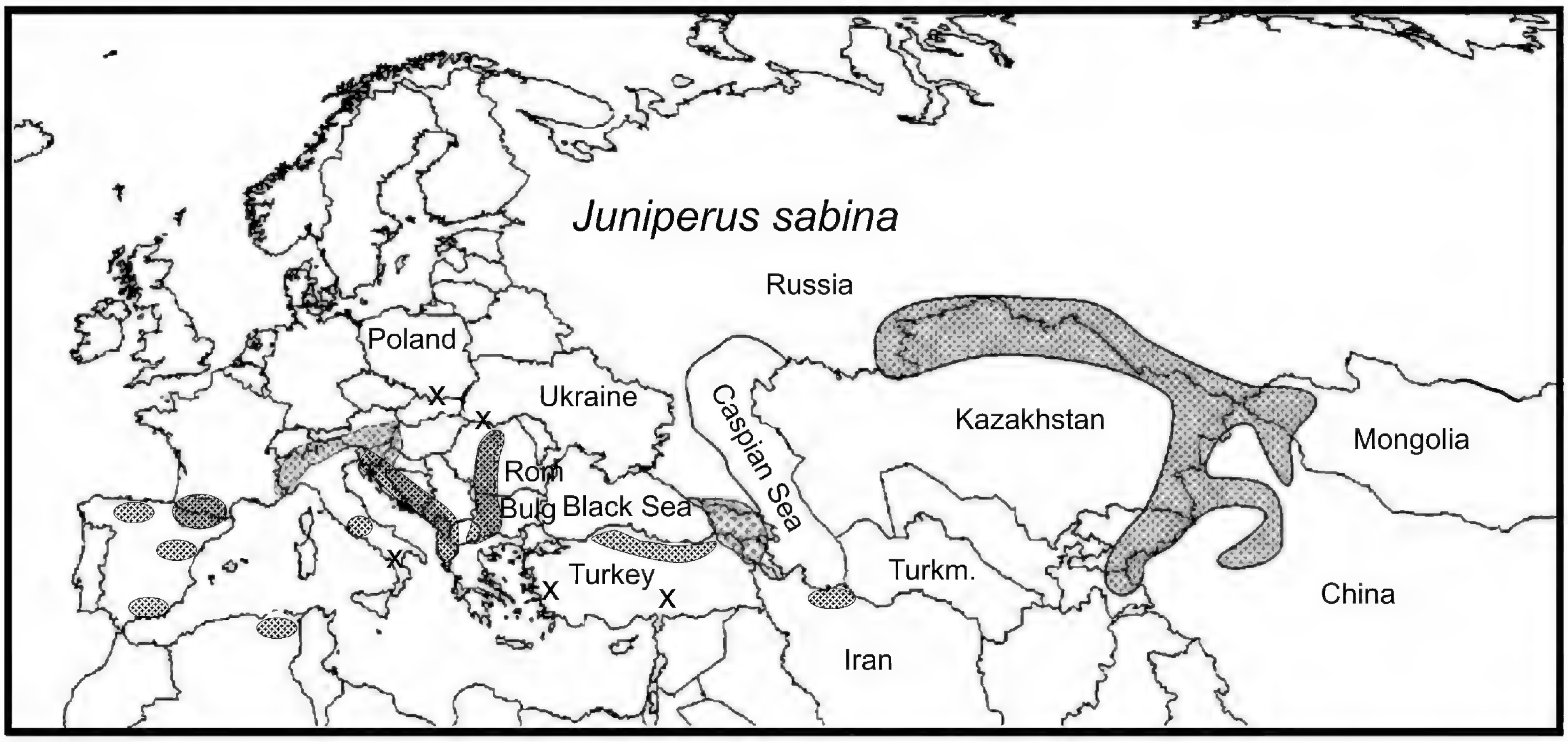

Fig. 1. Distribution (shaded areas) of *J. sabina*. x = outlying populations of *J. sabina*.

Adams et al. (2016) showed that nrDNA (ITS) did not resolve *J. sabina* populations due to the lack of sequence variation. However, their analyses (Adams et al., 2016) of cp DNA (petN-psbM, trnSG, trnDT, trnLF) revealed that *J. sabina* contained two kinds of cpDNA: typical *J. sabina* and that of *J. sabina* var. *balkanensis* cpDNA in a clade with *J. thurifera*. They recognized the taxon with the *J. thurifera* type cpDNA as a new variety: *J. s.* var. *balkanensis* R. P. Adams & A. N. Tashev. *Juniperus sabina* var. *balkanensis* was known only from sloping rocky limestone, at 1240 - 1630m, in the mountains of Bulgaria and northern Greece.

Subsequently, Adams et al. (2017), using samples from herbarium specimens, discovered two samples from far western Turkey that were *J. s.* var. *balkanensis*. However, numerous samples from throughout Europe were confirmed to be *J. s.* var. *sabina* (Fig. 2). Due to the proximity of the Macedonia - Bosnia-Herzegovina - Croatia region to the populations of *J. s.* var. *balkanensis* in Greece, it seemed prudent to make additional collections and analyses of *J. sabina* plants from these areas to more precisely determine the distribution of *J. sabina* var. *balkanensis*.

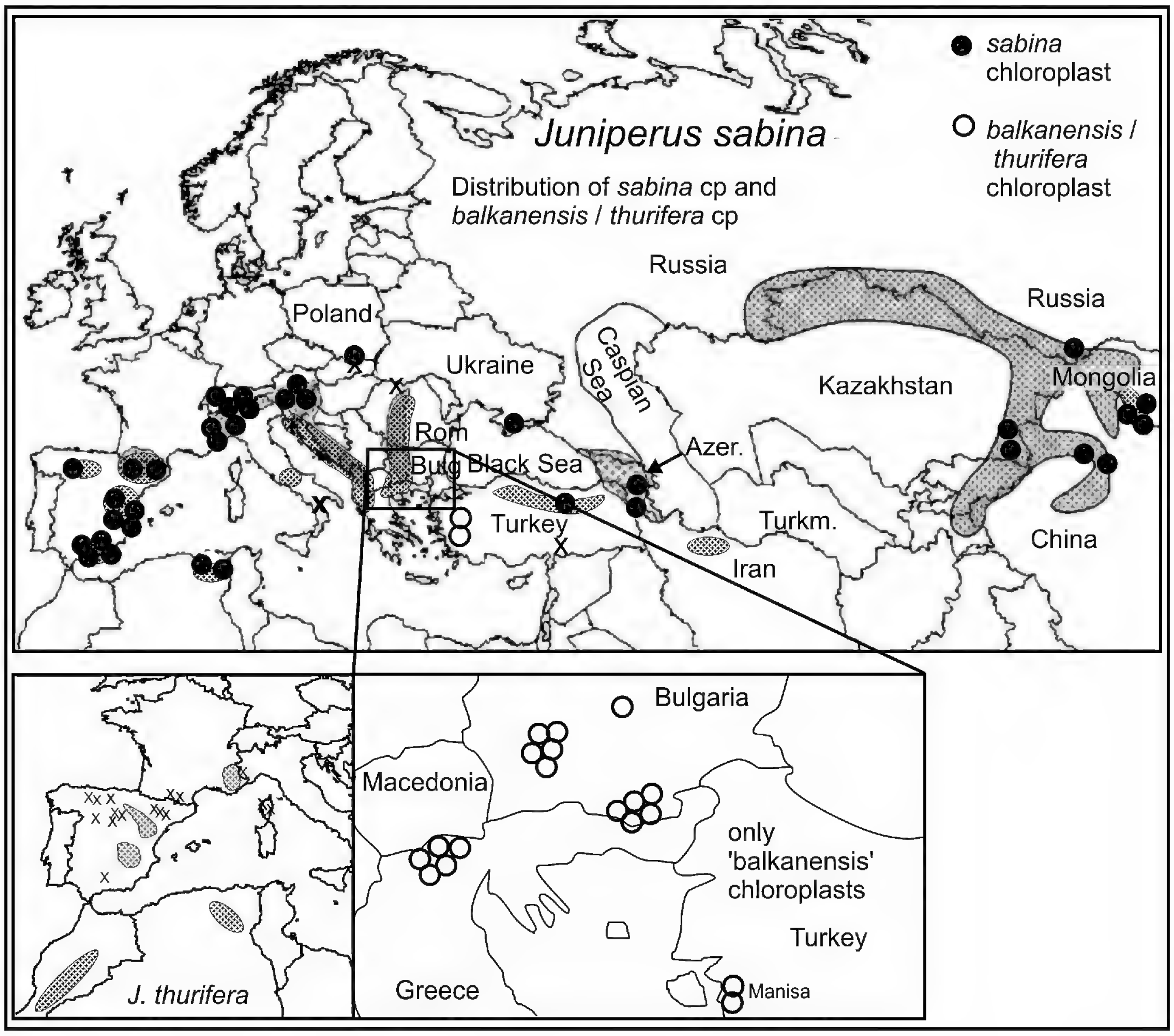

Figure 2. Distribution of *J. sabina* var. *balkanensis* and typical *J. sabina* chloroplast. The present day distributions of *J. thurifera* and var. *africana* (in north Africa) are shown in the insert on the lower left. (modified from Adams et al, 2017).

MATERIAL AND METHODS

Specimens used in this and previous studies: (species, popn. id., location, collection numbers): *J. chinensis*, CH, Lanzhou, Gansu, China, *Adams 6765-6767*; *J. sabina*: (SN), Sierra Nevada, Spain, *Adams 7197, 7199, 7200*; (PY), Pyrenees Mtns., Spain/ France border, *Adams 7573-7577*; (SW), Switzerland, *Adams 7611, 7612, 7614, 7615*; TS, Tian Shan Mtns., Xinjiang, China, *Adams 7836-7838*; Mongolia, Altai Mtns., *Adams 7585-7587*; Kazakhstan, Paniflor, *Adams 7811-7812*; Azerbaijan: *Adams 14316-14320*;

J. davurica (DV), 15 km se Ulan Bator, Mongolia, *Adams 7252, 7253, 7601*; *J. davurica* var. *arenaria* (AR) sand dunes, Lake Qinghai, Qinghai, China, *Adams 10347-10352*; river bank, Gansu, *J-Q. Liu and Adams 10354-10356*; *J. davurica* var. *mongolensis* (MS) sand dunes, 80 km sw Ulan Bator, Mongolia, *Adams 7254-7256*;

Collections of taxon with non-J. sabina cpDNA in Adams, Schwarzbach and Tashev (2016): (acronyms used in Fig. 7)

Bulgaria and Greece

B1-B5 Eastern Rhodopes. In protected site “Gumurdjinsky Snejnik”, locality “Madzharsky Kidik”. On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca* with *Juniperus communis*. 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m, 13 Aug. 2012, *Adams 13725-13729 (A. Tashev 2012-1-5*);

B6 Central Stara Planina (the Balkan). National Park “Central Balkan”. Reserve “Sokolna”. On a steep, rocky limestone slope, with *Sorbus aucuparia, S. aria, S. borbasii, Amelanchier ovalis, Carpinus orientalis, Sesleria latifolia, Pastinaca hirsute, Cephalanthera rubra, Laserpitium siler, Hieracium alpicola* etc. near a forest of *Fagus sylvatica.* 42°42'13.3" N,25°08'10.4" E, 1501 m , 22.08.2015. Bulgaria, *Adams 14721 (A. Tashev 2015 Balkan 1*;

B7-B9, Ba, Bb Rila Mountain, National Park “Rila”. On the eco-path, "Beli Iskar”, near river Beli Iskar, in a forest with *Pinus sylvestris, P. peuce, Picea abies, Abies alba, Juniperus communis, J. sibirica, Vaccinium myrtillus, Rosa canina, Sorbus aucuparia, Acer hyrcanum, Chamaespartium sagittale, Hypericum perforatum, Thymus* sp. etc. 42°14'26.5" N, 23°32'33.8" E, 1242 m, 24.06.2015. Bulgaria, *Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2)*;

G1-G5 Mt. Tsena, Greece, *Adams 14727-14731* (*A. Tashev 2015 So. 1-5 Tsena*);

Turkey

14861 Turkey, Manisa. Spil Daği Milli Parki (National Park) (Tas Suret), N38.55°, E 27.42°, ca 1250 m alt., leg. A. Boratyński, K. Boratyńska, 2005, TU_05/55, KOR 44573, female

14934 Turkey, Manisa, Spil Daği Milli Parki (National Park), N38°, 57', E 27° 41', 1024 m., *Tuğrul Mataraci 2016-1*

14938 Turkey, Gŭmŭshane, Kŭrtŭn, Aktas village, Karakaya (Northeast Anatolia), 40° 36' 03" N, 38° 53' 21" E., 2376 m. Coll. *A. Kandemir 10745.*

Samples new for this study: (with Lab Acc. ID = Adams xxxxx)

Bosnia-Herzogovian

Juniperus sabina var. *balkanensis*, on calcareous, dolomite, between Mt. Cvrsnica and Mt. Cabulja, 43° 34' 18.09" N, 17° 30' 39.88" E., 1460m, 7 June 2017, Bosnia-Herzogovina, Coll. F. Bogunic & Sonja Siljak-Yakovlev, Lab Acc. Robert P. Adams *15277-15281.*

Croatia

Juniperus sabina var. *balkanensis*, Mt. Biokovo, Vosac, Strbina, 1200 m, NB! 4n. 43° 18' 34.9" N, 17° 02' 36.3" E., 1300m, 15 June 2017, Croatia, Coll. Sonja Siljak-Yakovlev, Lab Acc. Robert P. Adams *15282-15286.*

Juniperus sabina var. *balkanensis*, 44°32'36" N, 15°10’09” E, 1080 m, fall 2017, Country: Velebit, Croatia, Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams *15343-15346.*

Macedonia

Juniperus sabina var. *balkanensis*, 41° 39' 18.16" N, 20° 44' 01.21" E., 4519 ft, Oct 2017, Macedonia, Mavrovo area. Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams *15311-15315.*

Juniperus sabina var. *balkanensis*, 41° 35' 46.61" N, 20° 39' 04.94" E., 4755 ft, Oct 2017, Macedonia, Gaichnik area, Coll. Katarzyna Marcysiak, Lab Acc. Robert P. Adams *15316-15320.*

Italy

Juniperus sabina var. *balkanensis*, P.N. del Pollino (San Lorenzo Bellizzi, CS), Timpa di San Lorenzo, su roccia calcarea, leaves from herbarium specimen [in Pisa, (PI)], 39.91349° N, 16.28578° E, 1436 m, 18 Aug 2017, Calabria, Italy, Coll. Francesco Roma-Marzio et L. Peruzzi, Lab Acc. Robert P. Adams *15365*

Juniperus sabina var. *balkanensis*. Valle di Selva Romana (Pennapiedimonte, Chieti), pendii rupestri, 1600-1900 m, 42° 07' 41" N, 14° 07' 18" W., 5411 ft, 01 Aug 2002, Abruzzo, Italy, Coll. F. Conti (APP No. 1701), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15413*

Juniperus sabina var. *balkanensis*. Vallone di Pennapiedimonte (Pennapiedimonte, Chieti), pascoli, 800-900 m, 42° 09' 01" N, 14° 11' 02" W., 2017 ft, 05 May 1999, Abruzzo, Italy, Coll. F. Conti (APP No. 13142), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15414*

Juniperus sabina var. *balkanensis*. Gran Sasso - M.te Camicia, loc. il Gravone (Castelli, Teramo), pendii rupestri, 1700 m, 42° 26' 45" N, 13° 44' 06" W., 5484 ft, 02 Nov 1996, Abruzzo, Italy, Coll. F. Conti (APP No. 17471), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15415*

Juniperus sabina var. *balkanensis*. M. S. Domenico (Pizzoferrato, Chieti), 41° 55' 28" N, 14° 14' 12" W., 3967 ft, 17 June 1995, Abruzzo, Italy, Coll. F. Conti (APP No. 33835), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15416*

Juniperus sabina var. *balkanensis*. Scatafosse (Villavallelonga, L'Aquila), 41° 47' 55" N, 13° 41' 13" W., 5809 ft, 01 Aug 2001, Abruzzo, Italy, Coll. F. Conti (APP No. 50172), ex Fabrizio Bartolucci, received 14 Apr 2018, Lab Acc. *Robert P. Adams 15417*

Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU), Herbarium (University of Forestry, Sofia, Bulgaria) and Herbarium Apenninicum (APP).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. 2.31 (Technelysium Pty Ltd.).

RESULTS

All the plants sampled in Macedonia, Bosnia-Herzegovina, Croatia and Calabria and Abruzzo areas of Italy were *J. s.* var. *balkanensis* based on cp DNA (Table 1). The revised distribution of v. *balkanensis* is shown in Figure 3. All known locations of v. *balkanensis* are in a relatively small geographical area.

Before discussing the nrDNA patterns, it should be noted that there are recent papers analyzing the inheritance of nrDNA in the Cupressaceae. Adams and Matsumoto (2016) analyzed 3 variable sites of nrDNA from synthetic crosses between *Cryptomeria japonica* cv. Haara and cv. Kumotooshi (= cv. Haara x Kumotooshi, ie., a backcross). They found that 3 of the 7 progeny had nrDNA very similar to that of the Haara x Kumo parent. In contrast, 4 of the 7 progeny had nrDNA exactly like the Haara parent. This appears to suggest that nrDNA polymorphisms can revert to that of a recurrent parent in the case of backcrossing. Adams, Miller and Low (2016) examined 8 variable nrDNA sites in the parents (*Hesperocyparis arizonica*, *H. macrocarpa*), and their 18 artificial hybrid progeny. Each of the 18 hybrids were heterozygous for all 8 nrDNA sites. This study is very relevant to the present study, because *Hesperocyparis* (= *Cupressus* in the western hemisphere) is very closely related to *Juniperus* (Little et al. 2004, Terry, et al. 2012, Terry and Adams, 2015, Terry et al. 2016) and because there are no verified artificial hybrids of *Juniperus* available to the authors for the examination of the inheritance of

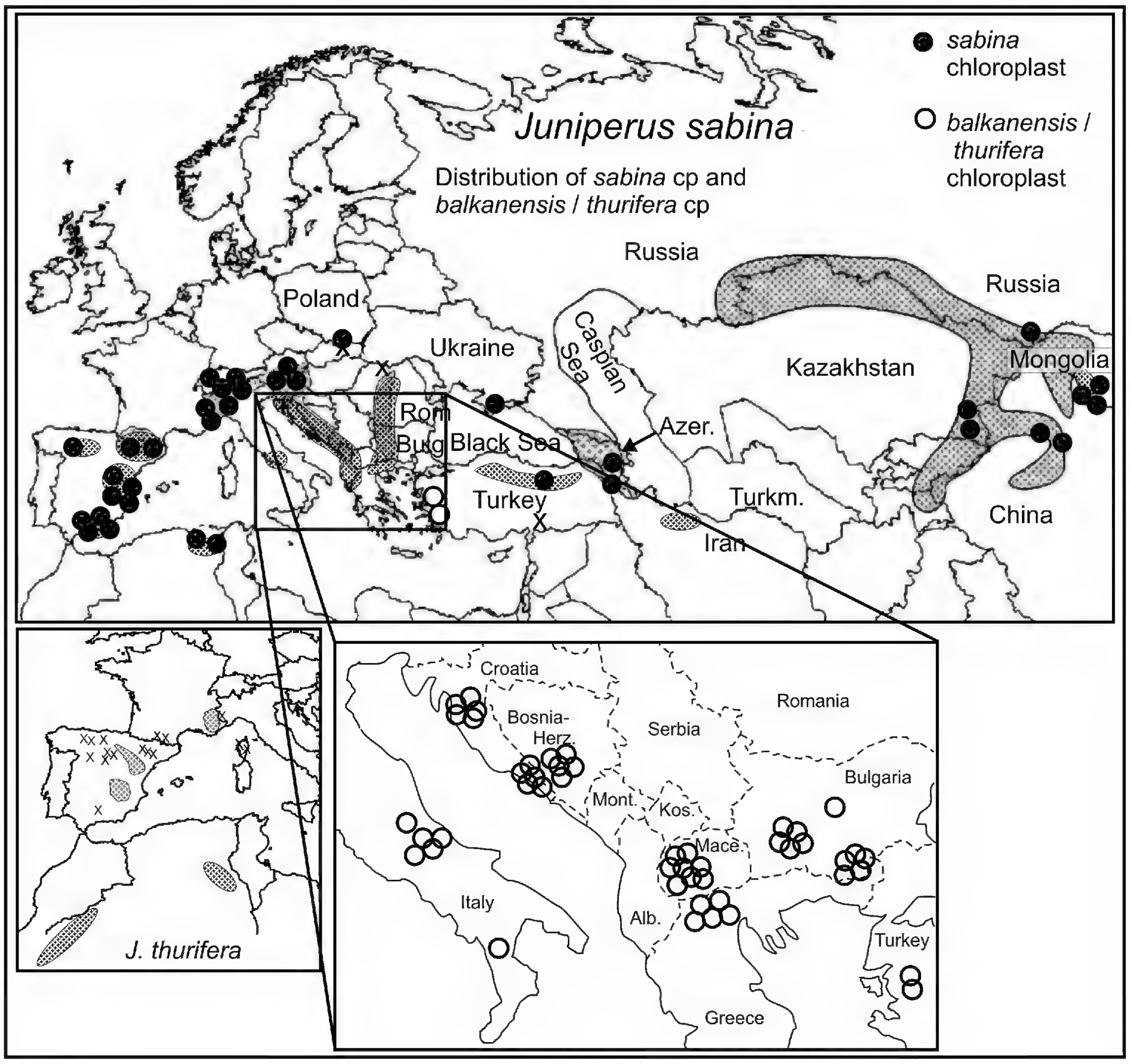

Figure 3. Revised distribution of *J. sabina var. sabina* (dark circles) and *J. s.* var. *balkanensis* (open circles) based on cp DNA (present study and from Adams et al. 2017)

nrDNA in *Juniperus*, the Adams, Miller and Low (2016) research on *Hesperocyparis* stands as a proxy for the inheritance of nrDNA in *Juniperus* and thus, their study in *Hesperocyparis* is surely applicable to *Juniperus*. So, we can confidently assume that nrDNA is inherited by complementation as found in *Hesperocyparis*, *Cryptomeria* and all other conifers. It should be noted that Adams, Miller and Low (2016) sequenced cp markers and confirmed that the cp genome is inherited via pollen (paternally inherited) in *Hesperocyparis* (and presumably *Juniperus*).

Aligning *J. sabina* and *J. thurifera* nrDNA sequences revealed that the taxa differ by SNPs at 22 sites, all in ITS1 or ITS2. However, a close examination of the nrDNA sequencing chromatograms revealed that only 8 of the 22 sites contained heterozygous peaks. The 8 sites were (with position): 352(R), 391(S), 432(R), 606(M), 785(Y), 999(M), 1046(R), 1047(K). Two nrDNA types were found considering these 8 sites. One nrDNA type (GGACCCAG) was found in 16/62 plants and type 2 (ACGACAGT) was found in 4/62 plants. The majority of the plants examined (42/62) were heterozygous for 1 to 8 sites (Table 1). Note that 33/ 42 heterozygous individuals were heterozygous for all 8 sites. The frequency of heterozygous sites was: 1,1,1,3,2,1,0,33 (for plants containing from 1 to 8 polymorphic sites, respectively). Heterozygous plants are somewhat randomly distributed (Fig. 4). It is interesting that 4/4 Spain and 2/3 Switzerland plants were homozygous for the 8 sites. Only 2/7 plants from the far East were homozygous. Plants of var. *balkanensis* seem to be a bit more heterozygous (only 12/48 were homozygous, Fig. 4).

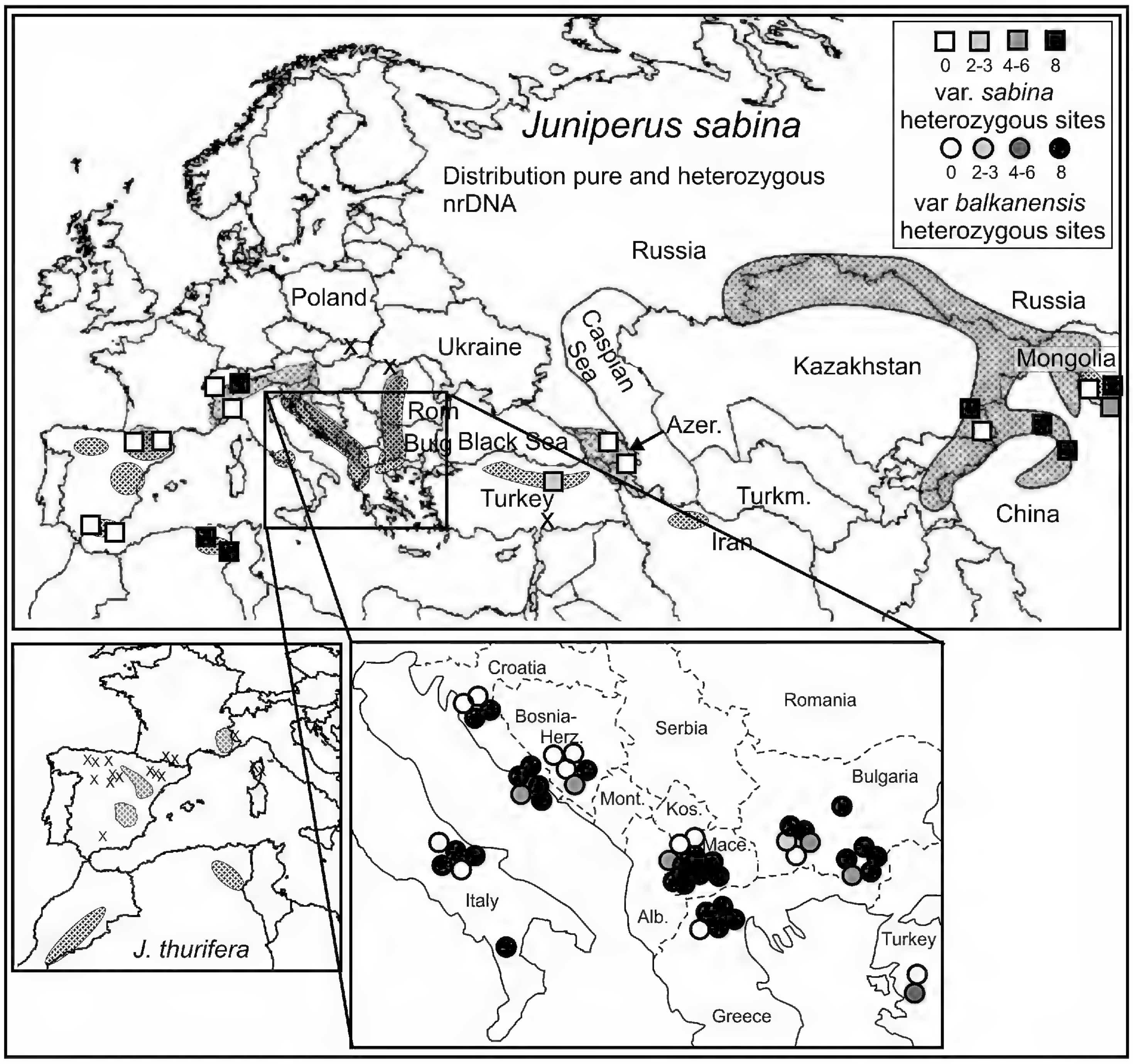

Figure 4. Distribution of homozygosity and heterozygosity among the 8 nrDNA polymorphic sites.

A more detailed examination of nrDNA type 1 (16/20) and type 2 (4/20) homozygous plants (Table 2) shows that both type 1 and type 2 plants were present in the Switzerland samples. One type 2 plant was found in Rila Mtn., Bulgaria and 2 were from Azerbaijan. All 16 type 2 nrDNAs share 4 bp sites with *J. thurifera*, and the four type 2 plants share 3 bp sites with *J. thurifera* (Table 2). It is strange that type 1 nrDNA contains 4 bp, typical of *J. thurifera* (in the 8 sites), and type 2 contain 3 different bp typical of *J. thurifera* (below and Table 2), but these are mutually exclusive in types 1 and 2.

most common sabina pattern (type 1)	G **G** A **C** *C* **C** A **G**	4 sites in common with *J. thurifera*
7083, thurifera, France, Morocco^2	**A G G C** ***T*** **C G G**	
2nd common sabina pattern (type 2)	**A** C **G** A *C* A **G** T	3 sites in common with *J. thurifera*

Crossing type 1 (GGACCCAG) x type 2 (ACGA*C*AGT) gives RSRM*C*MRK (see below), which differs at only site 5, from the putative hybrids (below and Table 1) of RSRMYMRK. Our data indicates that crossing between types 1 and 2 nrDNA types seem common, 42/62 plants were RSRMYMRK, however, none were RSRM*C*MRK! (the product of type 1 x type 2). These two nrDNA types may have arisen via hybridization with a *J. thurifera* ancestor and subsequent backcrossing to *J. sabina*.

most common sabina pattern (type 1)	G G A C C C A G
2nd common sabina pattern (type 2)	A C G A C A G T
cross between *J. sabina* type1 x type2	R S R M C M R K
Putative 'hybrid' pattern (table 1)	R S R M Y M R K

At our present level of understanding, the distributions of *J. s.* var. *balkanensis* and *J. thurifera* do not appear to overlap, negating modern hybridization. However, there were large changes in plant distributions in the Pleistocene and earlier, it seem probable that *J. thurifera*-like ancestors were sympatric with *J. sabina,* and presenting opportunities for chloroplast capture from *J. thurifera.*

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Table 1. Survey of *J. sabina* and classification based on ITS and trnS-trnG sequences. Putative hybrids and backcrosses [*J. sabina* x ancestor of *J. thurifera*] based on ITS polymorphisms are in **bold**.

coll. #, location	trnS-trnG classification (ie. cp genome)	ITS classif.	polymorphic sites[1]	ITS #poly/ 8 sites
			1 2 3 4 5 6 7 8	
14861 Spil Daği, Turk., Boratynski	**v. *balkanensis***	***sabina***	**R G R C Y C R G**	**4**
14934 Spil Dagi, Turkey, Mataraci	v. *balkanensis*	*sabina*	G G A C C C A G	0
13725 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**G G A M Y M R K**	**5**
13726 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13727 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13728 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13729 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14721 Sokolna reserve, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14722 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**G G A C Y C A G**	**1**
14723 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*	A C G A T A G T	0
14724 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**R G A C Y M A G**	**3**
14725 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14726 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14727 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14728 Tsena Mtn., Greece	v. *balkanensis*	*sabina*	G G A C C C A G	0
14729 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14730 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14731 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15311 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15312 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15313 Mavrovo, Macedonia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15314 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15315 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15316 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15317 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15318 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15319 Gaichnik, Macedonia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15320 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R C R A T M R K**	**5**
15277 Mt. Cabulja, Bosnia-Herze.	**v. *balkanensis***	***sabina***	**R S R A Y M R K**	**6**
15278 Mt. Cvsnica, Bosnia-Herze.	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15279 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	G G A C C C A G	0
15280 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	G G A C C C A G	0
15281 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	G G A C C C A G	0
15282 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15283 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**G G A C Y M R K**	**4**
15284 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15285 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15286 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15343 Velebit, Croatia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15344 Velebit, Croatia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15345 Velebit, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15346 Velebit, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**

15365 Calabria area, southern Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15413 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15414 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15415 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	G G A C C C A G	0
15416 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	G G A C C C A G	0
15414 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13167 Algeria	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
13168 Algeria	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7197 Sierra Nevada, Granada, Spain	v. *sabina*	*sabina*	G G A C C C A G	0
7199 Sierra Nevada, Granada, Spain	v. *sabina*	*sabina*	G G A C C C A G	0
7573 Sallent deGallego, Spain	v. *sabina*	*sabina*	G G A C C C A G	0
7574 Sallent deGallego, Spain	v. *sabina*	*sabina*	G G A C C C A G	0
7611 Switzerland	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7612 Switzerland	v. *sabina*	*sabina*	G C A C C C A G	0
7614 Switzerland	v. *sabina*	*sabina*	A C G A T A G T	0
14938 northeast Turkey Kandemir	**v. *sabina***	***sabina***	**R G R C T A G T**	**2**
14316 Azerbaijan	v. *sabina*	*sabina*	A C G A C A G T	0
14317 Azerbaijan	v. *sabina*	*sabina*	A C G A C A G T	0
7811 Kazakhstan, Paniflor	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7812 Kazakhstan, Paniflor	v. *sabina*	*sabina*	G G A C C C A G	0
7585 Mongolia, Altair Mtns.	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7586 Mongolia, Altair Mtns	**v. *sabina***	***sabina***	**A C G A Y M R K**	**4**
7587 Mongolia, Altair Mtns	v. *sabina*	*sabina*	G G A C C C A G	0
7836 China, Heaven Lake, Xinjiang	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7837 China, Heaven Lake, Xinjiang	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
most common sabina pattern			G **G** A C C C A G	0
7083, thurifera, France[2]			A **G** G C T C G G	0
9420, thurifera v. africana, Morocco[2]			A G G C T C G G	0
2nd most common sabina pattern			A C G A C A G T	0

[1] Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047.

[2] This pattern, **A G G C *T* C G G,** was also found in all *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, unpublished)

Table 2. *Juniperus sabina* classified based on ITS homozygous for all 8 polymorphic sites. Putative hybrids (heterozygous for the 8 polymorphic sites) were excluded.

coll. #, location	trnS-trnG classification	ITS classif.	nrDNA type	polymorphic sites[1] 1 2 3 4 5 6 7 8	# sites in common with *J. thurifera*
14934 Spil Dagi, Turkey, Mataraci	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
14728 Tsena Mtn., Greece	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15313 Mavrovo, Macedonia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15319 Gaichnik, Macedonia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15279 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15280 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15281 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15343 Velebit, Croatia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15344 Velebit, Croatia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
7197 Sierra Nevada, Granada, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7199 Sierra Nevada, Granada, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7573 Sallent deGallego, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7574 Sallent deGallego, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7812 Kazakhstan, Paniflor	v. *sabina*	*sabina*	1	G G A C C C A G	4
7587 Mongolia, Altair Mtns	**v. *sabina***	***sabina***	**1**	**G G A C C C A G**	**4, Note Type 1!**
7614 Switzerland	**v. *sabina***	***sabina***	**2**	**A C G A T A G T**	**3, Note Type 2!**
14723 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*	2	A C G A T A G T	3
14316 Azerbaijan	v. *sabina*	*sabina*	2	A C G A C A G T	3
14317 Azerbaijan	v. *sabina*	*sabina*	2	A C G A C A G T	3
7612 Switzerland	v. *sabina*	*sabina*	1	G C A C C C A G	3
				bases in common (**bold**) w *thruifera*	
most common sabina pattern(type 1)			1	G **G** A **C** *C* **C** A **G**	4 sites in common
7083, thurifera, France, Morocco[2]				**A G G C** *T* **C G G**	8
2nd common sabina pattern(type 2)			2	**A** C **G** A *C* A **G** T	3 sites in common
14861 Spil Daği, Turk., Boratynski	v. *balkanensis*	*sabina*		R G R C Y C R G	4
13725 eastern Rhodopes, Bulgaria	v. *balkanensis*	*sabina*		G G A M Y M R K	5
14722 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*		G G A C Y C A G	1
14724 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*		R G A C Y M A G	3
15320 Gaichnik, Macedonia	v. *balkanensis*	*sabina*		R C R A T M R K	5
15277 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*		R S R A Y M R K	6
15283 Mt. Biokovo, Croatia	v. *balkanensis*	*sabina*		G G A C Y M R K	4
14938 northeast Turkey Kandemir	v. *sabina*	*sabina*		R G R C T A G T	2
7586 Mongolia, Altair Mtns	v. *sabina*	*sabina*		A C G A Y M R K	4
most common sabina pattern(type 1)			1	G G A C C C A G	
2nd common sabina pattern(type 2)			2	A C G A C A G T	
cross between *J. sabina* type1 x type2			1x2	R S R M C M R K	
Putative 'hybrid' pattern (table 1)				R S R M Y M R K	

[1] Eight polymorphic sites (1-8): R352, S391, R432, M606, Y785, M999, R1046, K1047.

[2] This pattern, AGGCTCGG**,** was found in all *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, unpublished)

Composition of a Bottomland Forest on the Colorado River in the Lampasas Cut Plain of Texas

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ABSTRACT

We analyzed a hardwood bottomland forest along the Colorado River in the Lampasas Cut Plain of Texas and described species composition and structure of vegetation. Our analysis was conducted to provide baseline knowledge on the natural vegetation. The bottomland forest was comprised of three vegetational layers: 1) upper canopy of dominant trees including cedar elm (*Ulmus crassifolia*) and green ash (*Fraxinus pennsylvanica*), 2) under canopy of heavily browsed lianas, shrubs, and shorter trees including saw greenbriar (*Smilax bona-nox),* Texas persimmon (*Diospyros texana*), and western soapberry (*Saponaria saponaria*) as well as 3) an herbaceous zone of Canada wild rye (*Elymus canadensis)* and Texas wintergrass (*Nasella leuchotricha*), sedges, as well as both annual and perennial forbs. Cedar elm and green ash were dominant trees but had little regeneration, which may be caused by hydrological differences, grazing by cattle, as well as heavy browsing by white-tailed deer (*Odocoileus virginianus*) and felling and girdling by American beavers (*Castor canadensis*). In addition we compare vegetation with high importance and dominance as well as diversity to other rivers found in the West Cross Timbers of Texas. Published on-line www.phytologia.org *Phytologia 100(2): 128-144 (Jun 22, 2018).* ISSN 030319430.

KEY WORDS: Bottomland forest, Colorado River, Lampasas Cut Plain, Plant community ecology

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Bottomland forests are some of the most widely distributed, biodiverse, and productive of communities throughout southern regions of North America (Braun 1964; Messina and Conner 1998; Baker et al. 2004). Diamond et al. (1987) reported that bottomland forest dominated by sugarberry (*Celtis laevigata* var. *laevigata*), cedar elm (*Ulmus crassifolia*), and pecan (*Carya illinoinensis*) comprised the most widely distributed forest community in Texas. It has been estimated that over one-half of the bottomland forest ecosystem in Texas has been lost (Barry and Kroll 1999) and many, including the Colorado River in this investigation, have had their hydrology changed due to damming (Texas Parks and Wildlife (TPWD) 2012). Because of these losses there is considerable interest in restoration of bottomland forest and preserving remaining tracts of less-altered bottomland forests. However, little is known about community composition of bottomland forests in Texas.

Description of the natural vegetation is an important phase of preservation and restoration of bottomland forests. To date there has been limited description and vegetational analysis of such forest communities (TPWD 2012). The Society of American Foresters (SAF) included qualitative coverage of these forests in its descriptions of forest cover types (Eyre 1980) and the Society for Range Management (Shiflet 1994) excluded most forest range types. Neither group examined understory quantitatively. There are several bottomland forest cover types in the southern region that have relatively open canopies and well-developed lower layers of vegetation including two recognized by the SAF (Eyre 1980): SAF 93, sugarberry-American elm (*Ulmus americana*)-green ash (*Fraxinus pennsylvanica*) and SAF 94, sycamore (*Platanus occidentalis*)-sweetgum (*Liquidambar styraciflua*)-American elm. The latter was previously designated as the sycamore-pecan (*Carya illinoinensis*)-American elm type (Eyre 1954).

Later descriptive studies and subsequent qualitative reports of southern floodplain forests (Diamond et al. 1987; Meadows and Stanture 1997; Twedt and Best 2004; Lockhart and Kellum 2006; Twedt et al. 2010) indicated the widespread sugarberry-elm-pecan forest type to be highly variable in its composition, especially where it is ecotonal to adjacent cover types. The general forest community as found in Texas was described variously as elm/sugarberry parks/woods (McMahan and Frye 1987), sugarberry-elm series (Diamond et al. 1987), sugarberry-elm floodplain forest (Bezanson 2000), and Edwards Plateau floodplain hardwood forest (Elliott 2013). Rosiere et al. (2013) described a form of the sugarberry-cedar elm-pecan forest along the Bosque River in North Central Texas, which aligned with the description of southern floodplain forests described above.

Descriptions of understories of woodlands in eastern and southern forest regions of the United States commonly have been in conjunction with soil surveys under leadership of the Natural Resources Conservation Service (NRCS) as outlined in national range handbooks (Soil Conservation Service 1967, 1976; NRCS 2003) and, more recently, river authorities (Jones-Lewey 2016). In an attempt to generally describe riparian areas across the state, the Nueces River Authority (NRA) produced a field guide, which included some of the common vegetation found in Texas riparian areas (Jones-Lewey 2016). Descriptions of grazeable woodlands are currently written as forest land ecological sites (NRCS 2003). Forest land ecological site descriptions need greater detail regarding forest vegetation, including that of the understory. Likewise, classification of natural communities such as forest alliances and series (Diamond et al. 1987; McMahan and Frye 1987; Bezanson 2000, Hoagland 2000) as well as the field guide by the NRA (Jones-Lewey 2016) have been largely qualitative with limited quantitative information provided.

There have been no published, quantitative descriptions of herbaceous plants occurring along the Texas Colorado River. In an investigation examining mammal habitats, Scales and Wilkins (2003) assessed two riparian transects in Colorado Bend State Park, which occur along the Colorado River in the adjacent Edwards Plateau ecoregion. They identified woody plants in the riparian woodlands as pecan, green ash, black walnut (*Juglans nigra*), plateau live oak (*Quercus fusiformis*), red oak (*Q. rubra*), and American elm but did not quantify the understory.

We conducted the current study to provide descriptions and analyses of bottomland forests of the Texas Colorado River in an ecotonal area between the West Cross Timbers and the Edwards Plateau called the Lampasas Cut Plain (Diggs et al. 1999) and compare it to similar studies conducted in the West Cross Timbers of Texas. Currently there is a need for quantitative data of this forest vegetation, which is lacking for much of Texas (Diamond et al. 1987) and because of ongoing classification and ground-truthing of natural plant communities (Elliott 2013), as well as riparian restoration projects. This investigation provided the first quantitative data for bottomland forests in the Lampasas Cut Plain of Texas.

MATERIALS AND METHODS

The study area was within the Lampasas Cut Plain ecoregion (Diggs et al. 1999) and Cross Timbers and Prairies vegetational area (Correll and Johnston 1979) in Mills County, Texas (Figure 1). The Colorado River in Texas is the longest river confined to the state, which begins in the Caprock Escarpment of the high plains near Lamesa, Texas, and flows to the Gulf of Mexico at Matagorda Bay (Crisp 2012). The specific locality for the investigation was the Timberlake Biological Station near Goldthwaite, Texas downstream from Lake O. H. Ivie, which was constructed 27 years ago at the confluence of the Concho and Colorado rivers (Williams 2016), about 145 km south of Abilene, Texas. This tract had been subjected to grazing by livestock for ≥ 100 years. A resident herd of white-tailed deer (*Odocoileus virginianus*) occurred in the study area. There was evidence of heavy browsing (felling and girdling) by American beavers (*Castor canadensis*). We sampled vegetation from August 30, 2014 to September 26, 2014 using nonpermanent plots, which have been shown to yield few statistically significant differences from permanent plots in riparian vegetation monitoring (Laine et al. 2013).

We used the step-point method (Evans and Love 1957; Bonham 1989) to determine composition of herbaceous and seedling (< 1.0 cm in diameter) species from the bottomland. Plants were sampled randomly with a sharp-pointed pipe and total and relative numbers of hits were recorded. We sampled a total of 3800 points in the bottomland within six rectangular quadrants each of which was 25 by 50 m with the longest dimension parallel to the river bank as described by Ford and Van Auken (1982) and Wood and Wood (1988, 1989). The six areas were sampled on the north side of the Colorado River in two areas that included about 2 km of forested area adjacent to the river. Three samples within the 1.0 km stretch were taken on both sides of an elevated area cut by a rill that entered the river.

For woody vegetation in the bottomland, we used the same six rectangular quadrants, 25 by 50 m to sample all woody species greater than 1.0 cm in diameter. We identified the woody species and measured diameter at breast height (dbh). The dbh was used to calculate basal area. We calculated density (plants/ha), dominance (basal area/ha), and relative-importance values as described by Ford and Van Auken (1982) and Wood and Wood (1988, 1989). Shannon diversity, richness, and evenness were calculated according to formulas in Ludwig and Reynolds (1988).

We also assessed beaver damage at the sites that we sampled. This was accomplished by counting trees that were gnawed, girdled, or downed in the 25 by 50 m bottomland quadrats. The species damaged by beaver were identified and percentages for tree species recorded.

Species of plants were identified and classified using Diggs et al. (1999), which also served as the reference for common and scientific names. We deposited voucher specimens in the herbarium (TAC) at Tarleton State University in Stephenville, Texas. Using classifications for the Great Plains ecoregion, wetland indicator status for plants was obtained from Lichvar et al. (2016).

RESULTS

The bottomland forest was comprised of three vegetational layers: 1) upper canopy of dominant trees including cedar elm and green ash, 2) under canopy of heavily browsed lianas, shrubs, and shorter trees including saw greenbriar (*Smilax bona-nox),* Texas persimmon (*Diospyros texana*), and western soapberry (*Saponaria saponaria*) as well as 3) an herbaceous zone of Canada wild rye, Texas wintergrass (*Nasella leuchotricha*), sedges, as well as both annual and perennial forbs.

There were 13 species of trees sampled in this forest of which all were native. For all woody species >1.0 cm in diameter, cedar elm had the highest relative-importance value (70.7) and greatest dominance as well as the greatest relative cover >1.0 cm of any species of tree (Table 1). In all measured attributes, cedar elm was the most common tree. After cedar elm, the most common trees, >1.0 cm overall, were green ash and western soapberry (Table 1). Most of the tree species had a wetland indicator status of facultative (Table 1).

There was a total of seven species of shrubs and one liana. The most common species of shrub was Texas persimmon (Tables 1 and 2). Prickly-pear cactus (*Opuntia engelmannii* var. *lindheimeri*) was also common on one relatively high bank and occurred in two large clumps. More widely distributed and relatively high in importance was chittamwood (*Sideroxylon languinosum* subsp. *oblongifolium*). Saw greenbriar (*Smilax bona-nox*) was the only liana and accounted for less than 2% of vegetation sampled that was <1.0 cm in diameter. Most of the shrub species had a wetland indicator status of facultative or upland (Table 1). There were no introduced tree or shrub species in the quadrats. However, Chinaberry (*Melia azedarach*) and chastetree (*Vitex agus-castus*) were occasionally observed outside the quadrats.

Of the herbaceous species, 23 were native and five were introduced (Table 2). Native perennial grasses comprised much of the herbaceous vegetation in the floodplain and were dominated by Canada wildrye and Texas wintergrass. Four forbs, pigeonberry (*Rivinia humilus*), betony noseburn (*Tragia betoncifolia*), cowpen daisy (*Verbesina encelioides*), and introduced horehound (*Marrubium vulgare*) were relatively common and generally more abundant than other forbs (Table 2). Most herbaceous species had a wetland indicator status of upland or facultative upland (Table 2). Spiny-aster (*Chloracantha spinosa*) was the only facultative wetland species (Table 2). There was little regeneration of woody vegetation as evidenced by only 3.4% of total trees and shrubs being < 1.0 cm in diameter (Table 2). Honey mesquite, Texas persimmon, and western soapberry had the greatest percentages of small saplings (Table 2).

Beaver damage was greatest on the cedar elms, with 67% of damage occurring on cedar elm trees (Table 3). Richness, Evenness, and Shannon Diversity in the Texas Colorado River Bottomland, when compared to the Bosque River in the nearby West Cross (Rosiere et al. 2013), was lower than the Bosque River, except in the case of richness of herbaceous and seedling species (Table 4).

DISCUSSION

Quantitative data for woody and herbaceous vegetation in bottomland forest of the Lampasas Cut Plain, including nonnative species, were provided for the first time. Comparison of woody and herbaceous vegetation to other studies near the region, possible successional status within the bottomland forest, herbivory, hydrology, and diversity are discussed.

Little has been published regarding woody vegetation along the Colorado River. Scales and Wilkins (2003) identified the following trees occurring along two transects along the Colorado River in Colorado Bend State Park: pecan, green ash, black walnut, plateau live oak, (red oak), and American elm. At Timberlake Biological Station, we did not sample black walnut or oaks in the bottomland and found much more cedar elm than American elm (Table 1). Patterns of density and importance values for cedar elm along the Texas Colorado River corresponded to those reported for similar forested communities in the region. Composition and structure of the forest along the Texas Colorado River was similar to various woody communities in the Trinity River basin of northern Texas as described by Nixon et al. (1990) and an old-growth bottomland forest along a creek in northeastern Texas (Nixon et al. 1991). In these forests cedar elm and sugarberry were consistently common or dominant trees. Nixon et al. (1991) reported that in a creek-floodplain forest, cedar elm and sugarberry had highest importance values and greatest densities of trees. Duke (2015) pointed out that sugarberry can become invasive when riparian functionality is compromised and may replace key riparian indicator species such as green ash, cottonwood, and black willow. We did not find this to be the case at this site on the Colorado River with cedar elm and green ash being more dominant than sugarberry.

Members of the elm family, like sugarberry, American elm, and cedar elm, occurred in the bottomland (Table 1) and sugarberry is reported to be frequently browsed by ungulates and its fruits are an important food source for many birds (Linex 2014). Linex (2014) and Jones-Lewey (2016) stated that elms are the most widespread and important riparian trees in Texas, which help protect river banks during flooding. Linex (2014) indicated that American elm and cedar elm are frequently browsed by cattle and deer. In our sampling quadrats, sugarberry and elm trees had poor regeneration (Table 2) and if they survived past the seedling stages, small trees were often felled or girdled by beaver (Table 3).

Green ash has relatively extensive coverage across East, Central, and South Texas and is highly tolerant of disturbance growing not only along the streamside but on extremely steep channel slopes (Duke 2015). Jones-Lewey (2016) indicated that green ash is important in protecting banks during floods and one of the most common species of ash in the eastern one-third of Texas. Linex (2014) added that it provided fair browse value for deer and was one of the first trees to grow back in abandoned fields adjacent to or replacing bottomlands. Green ash was the second most important tree species at our study sites but had little regeneration (Tables 1 and 2). Grazing and browsing likely accounted for the lack of green ash regeneration in the Colorado River bottomland. The only common regenerating small trees or shrubs were honey mesquite, Texas persimmon, and western soapberry. Texas persimmon is reported to be only occasionally browsed by cattle and deer, honey mesquite provides fair browse value, and western soapberry is readily browsed by cattle and deer (Linex 2014). Lastly, saw greenbrier, which has a stem diameter <1.0 cm, was relatively common (Table 2) but not as common as that found along the Bosque River (Rosiere et al. 2013), which had not been grazed by cattle in over 50 years. It is reported to be readily browsed by herbivores and its fruits are important to a variety of wildlife (Linex 2014).

TPWD (2012) and Nelle (2015) listed Chinaberry (*Melia azedarach*), Chinese tallow (*Sapium sebiferum*), Japanese honeysuckle (*Lonicera japonica*), and salt cedar (*Tamarix* spp.) as nonnative species that could be problematic in bottomlands associated with the Colorado River in the Lampasas Cut Plain. TPWD also listed tree of heaven (*Ailanthus altissima*) as an invasive tree. Anderson (2006) listed Chinaberry and chastetree as non-native species found in the river corridor near Austin and Bastrop, Texas. Only two species of nonnative woody plants, Chinaberry and chastetree, were observed in the bottomland forest at the study site but these were not sampled in the six quadrats, because they were not common. Chastetree likely escaped from yards near the river. Chinaberry was a rapid-growing species along the San Antonio River (Bush and Van Auken 1984) and a species associated with sugarberry and cedar elm (Van Auken and Bush 1985). Richardson et al. (2007) explained that rivers were very susceptible to invasion by alien plants because hydrologic dynamics and frequent disturbances of streams make them especially effective for dispersal of plant propagules. Bush and Van Auken (1984) commented that Chinaberry along with sugarberry and native colonizing tree species likely became established following flooding. Chinaberry may be considered invasive and spreads rapidly along riparian areas (Jones-Lewey 2016). To date, it is only a minor component of the woody vegetation at this study site.

Previous investigations, as well as qualitative descriptions of bottomland forests (Bush and Van Auken 1984; Diamond et al. 1987; McMahan and Frye 1987; Bezanson 2000), generally placed less emphasis on shrubs and provided little data and analysis of herbaceous layers of bottomland-hardwood forests. By contrast, this investigation included the understory of the forest. Density and dispersion of trees combined with the absence of lianas and relatively low number of seedlings and saplings of trees formed a canopy sparse enough for development of an herbaceous understory dominated by native perennial grasses along the Colorado River. Herbaceous layers of the forest along Colorado River were similar to those reported for bottomland forests of the West Cross Timbers in Texas (Rosiere et al. 2013). Nixon et al. (1991) reported about the same number of shrubs for a creek-bottom forest, but most of these shrubs, other than woody vines, were absent from the forest adjacent to the Colorado River.

Canada wildrye and Texas wintergrass were herbaceous co-dominants in the bottomland forest but in a quadrat sampled in an area that had not been grazed and was adjacent to the quadrats in this study (Nelson unpublished data), broadleaf woodoats were more common. Broadleaf woodoats are reported to stabilize soils on steep banks (Jones-Lewey 2016), and to be grazed as well as browsed (Linex 2014). Loss of broadleaf woodoats (*Chasmanthium* latifolium)by being grazed and browsed out of the bottomland contributes to erosion of river banks. Canada wildrye and broadleaf wood oats were dominants in a West Cross Timbers bottomland (Rosiere et al. 2013) and broadleaf woodoats is common on the floodplains along rivers in the Texas Hill Country (Gustafson 2015). They are viewed as dominants in late-seral to climax vegetation along streams and floodplains throughout much of Oklahoma (Tyrl et al. 2008) and Texas (Gould 1975). Canada wildrye is reported as excellent forage for livestock and is browsed by deer when young but can become susceptible to overgrazing (Linex 2014; Jones-Lewey 2016). Texas wintergrass was an upland plant that occurred mostly in elevated areas of the study sites. It provides fair to good grazing for cattle and fair browse for deer (Linex 2014). There were relatively few species of sedge (*Carex*) and umbrella sedge (*Cyperus*) sampled (6% of total), which are often wetland species, and may indicate negative changes in hydrology when reduced or absent (Jones-Lewey 2016).

TPWD (2012) listed King Ranch bluestem (*Bothriochloa ischaemum* var. *songarica*) and Bermuda grass (*Cynodon dactylon*), as nonnative species that could be problematic in bottomlands associated with the Colorado River in the Lampasas Cut Plain. There were four introduced grasses with Bermuda grass being the most common on the Colorado River (Table 2). Bermuda grass, which comprised 5% of the total herbaceous coverage provided some stability for river banks but often out-competed native plants, which frequently provided greater bank stability (Jones-Lewey 2016). Bermuda provided good forage for livestock but not deer and is known to survive saturation by flooding for up to three weeks (Linex 2014). We did not sample King Ranch bluestem in our bottomland quadrats but observed it in the uplands adjacent to the bottomland.

Pigeonberry, betony noseburn, and cowpen daisy were relatively common native forbs that were facultative or upland classified plants and often in environments that were recently disturbed. Pigeonberry is reported to occur in stream bottomland woods (Diggs et al. 1999) in shaded areas (Gustafson 2015), which was where it occurred on the Colorado River. Linex (2014) states that it is occasionally grazed by cattle and browsed by deer and is likely spread into bottomlands by birds (Linex 2014). Betony noseburn is common on sandy soils (Diggs et al. 1999), which were common along the Colorado River. Cowpen daisy is known from sandy soil in many disturbed habitats (Diggs et al. 1999; Gustafson, 2015), which was where it was more abundant on the Colorado River. Pigeonberry is known from stream bottomland woods (Diggs et al. 1999) and prefers shaded areas (Gustafson, 2015), like the bottomland woods on the Colorado River. A fourth forb, spiny-aster (*Chloracantha spinosa*) was the only facultative wetland species. Its rhizomes help stabilize river banks and young plants are eaten by deer and cattle (Linex 2014).

Frostweed was not common along the Colorado River (Table 2). Frostweed is viewed as a species typical of middle stages of succession (Tyrl et al. 2008), but a study (Thompson and McKinney 2006) of various successional stages in a river bottomland forest in Tennessee with similar composition and structure to the one reported herein, reports frostweed to be associated with higher seral states. In the Colorado River bottomland, frostweed was often associated with perennial grasses in more open spots within the herbaceous layer. Its lack of abundance in this investigation might be due to the Colorado River herbaceous layer being at an earlier stage of succession or to differences in herbivory (Linex 2014).

The only introduced forb on the Colorado River was horehound, which often grows near cow lots or under shade where cattle and other livestock such as sheep and goats congregate and the soil has high nutrient levels (Diggs et al. 1999). In the Colorado River bottomland sampled, disturbed areas caused by cattle were mainly where horehound was sampled.

In terms of bottomland forest succession along the Colorado River, we concluded that the cedar elm-green ash community was representative of the potential natural vegetation (Zerbe 1998) or climax floodplain forest (Braun 1964) for this ecological site. Composition and structure was consistent with traditional descriptions ranging from that of forest cover types (Eyre 1980) to classification for conservation of plant communities (Diamond et al. 1987; Hoagland 2000). This was in contrast to Holcomb (2001) that suggested cedar-elm-green ash as the mid-successional stage.

Holcomb (2001) used the qualitative and generic pattern that Hodges (1997) proposed for bottomland forests of the Atlantic and Gulf Coastal Plains and it was not clear how relevant the general coastal model of Hodges (1997) would be for the inland Lampasas Cut Plain ecoregion. The forest investigated by Barry and Kroll (1999) and Holcomb (2001) was along the Elm Fork of the Trinity River in the more mesic East Cross Timbers, whereas the forest along the Colorado River occurred in the more arid Lampasas Cut Plain (Diggs et al. 1999).

Quantitative studies of bottomland hardwood forests have been less common than qualitative analyses. Furthermore, quantitative investigations generally were limited to woody species as, for example, those of similar bottomland forests along the San Antonio River (Bush and Van Auken 1984; Van Auken and Bush 1985), Trinity River (Nixon et al. 1990), and Spring Creek in the Blackland Prairie (Nixon et al. 1991). Barry and Kroll (1999) concluded that a sugarberry-cedar elm-green ash bottomland forest was the climax community for a similar bottomland habitat. Nixon et al. (1991) reported that green ash was a dominant tree along with species of elm, pecan, sugarberry, and species of oak. Similarly, green ash was the second most important species in the forest of our study.

Pecan was an uncommon species in our investigation and was not reported as being highly important by Barry and Kroll (1999) or Rosiere et al. (2013). Holcomb (2001) conducted studies following those of Barry and Kroll (1999) on the same tract and concluded that pecan was a dominant of the seral stage that preceded the sugarberry-elm-green ash community. This was consistent with the sugarberry-cedar-elm-pecan community that Rosiere et al. (2013) described, in which pecan was, perhaps, a subclimax species that persisted into the climax forest.

Van Auken and Bush (1985) studied secondary succession of a climax forest on terraces of the San Antonio River and explained that sugarberry and cedar elm became dominant in advanced stages of development of forests, whereas pecan established earlier in the successional sequence and persisted into the climax forest. The few pecans we encountered on the Colorado River were small and not larger, older trees such as were sampled along the Bosque River (Rosiere et al. 2013). Limited recruitment of pecan as one of the dominant species was consistent with Rice (1965). It appeared that sugarberry and cedar elm were the climax dominants, while pecan was a member of the climax as a persistent subclimax species (Rosiere et al., 2013). Burns and Honkala (1990) categorized pecan as subclimax. Perhaps pecans have only recently become established along this portion of the Colorado River and/or old trees have died. Pecan provides poor to fair browse value for deer (Linex 2014), but it was low in numbers of < 1.0 cm samples taken along the Colorado River. However, because browse value of pecan is poor to fair at best (Linex 2014), it seems unlikely that low pecan regeneration was due to herbivory.

Braun (1964) and Kellison et al. (1998) concluded that sugarberry is nearly ubiquitous in bottomland forests throughout the south-central region, whereas cedar elm is more restricted, being confined primarily to western parts of this region. The large amount of cedar elms along the westward Colorado River supported this hypothesis. According to Wagner (2003), there were three types of dominant overstory plant communities in the limestone-dominated Central Texas/Edwards Plateau region: bald cypress (*Taxodium distichum*) and sycamore (*Platanus occidentalis*); pecan and sugarberry; and sugarberry and elm. Based on these two observations, the bottomland forest on this portion of the

Colorado River is more like forests in the Cross Timbers ecoregion of Texas and was less like Edward's Plateau sites near Bastrop and Austin, Texas (Anderson 2006).

We also concluded that herbivory is likely affecting regeneration of woody species and perennial herbs in the Colorado River bottomland. Beaver are not common in the Hill Country (Gustafson 2015) but appear to be common and detrimental at this study site on the Colorado River in the Lampasas Cut Plain. They can occur in burrows in river banks (Wilson and Ruff 1999; Schmidly 2004) rather than in lodges generally located away from banks, which was the case at the Colorado River study site. Because of their extensive modification of freshwater environments, beaver may be considered keystone animals as ecosystem engineers in many areas (Jones et al. 1994; Wilson and Ruff 1999; Karklins 2017); however, when they become too abundant and act as a dominant in the ecosystem, they have caused negative changes in the bottomland ecosystem (Townsend and Butler 1996; Gibson and Olden 2014; Karklins 2017). We observed only small amounts of cottonwood or willow in our quadrats indicative of a possible preference of these species by beaver. Beaver eat bark and leaves of many shrub and tree species but cottonwood and willow are preferred (Wilson and Ruff 1999). Small et al. (2016) reported that in northern New Mexico, the most important plant variable for the presence of beavers was willows (*Salix* spp.) and that grazing by cattle, as currently practiced on Forest Service grazing allotments, disrupted the beaver-willow mutualism, rendering streams unsuitable for beaver. The scarcity of willows and cottonwoods in the bottomland may be due to heavy grazing by cattle, browsing by white-tailed deer, and large numbers of American beaver, or lack of bottomland flooding. However, willows and cottonwoods did occur more often in the near-riparian zone, 2.5 m from the water's edge (Nelson unpublished data), which is steep and therefore not as accessible for herbivores. Black willow and cottonwood provide good browse and is often heavily used by cattle and deer (Linex 2014). Additional investigations into the role of beaver in these bottomlands is needed.

Improperly managed white-tailed deer can cause significant damage to riparian vegetation by their consumption of forbs and shrubs as browse (Nelle 2015). White-tailed deer were observed in the Colorado River bottomland frequently. Nelson Dickinson and Van Auken (2016) reported that large vertebrate herbivores, mainly white-tailed deer, significantly affected the survival and density of juvenile bigtooth maple (*Acer grandidentatum*) at Lost Maples State Park in Texas. Cogger et al. (2014) tabulated that at bottomland forest restoration sites along the Upper Mississippi River and its tributaries, white-tailed deer browsed 46% of tree seedlings and preferred American elm over green ash.

Livestock grazing has affected almost all riparian areas in the state and is considered one of the most significant disturbances affecting them (Nelle 2015). Removal of cattle from riparian areas in the Northwestern Great Basin resulted in dramatically increased coverage in riparian vegetation (Batchelor et al. 2015). Also, cattle are reported to play a role in mesquite seed dispersal (Ansley et al. 2017), and may have contributed to its higher regeneration in the quadrats at our study site. Nelle (2005a; 2005b) concluded that heavy grazing, watering, and loafing by cattle damages riparian vegetation, generally leading to destabilized river banks. One instance of beneficial impacts reported for cattle grazing was that ephemeral wetland diversity increased with cattle grazing, which removed exotic grasses from the wetlands (Marty, 2005). In the stretch of the Colorado River bottomland sampled, there were few to no ephemeral wetlands and our data suggested that herbivory by cattle, deer, and beaver activity may have impeded regeneration of trees and shrubs (Table 2).

Another possibility for reduced regeneration of woody species and herbaceous perennials is low flow hydrology. Low flow hydrology during droughts and flood flow events have been documented in changing riparian vegetation (Hardy and Davis 2015). Most bottomlands have been disrupted by dams, which separate and isolate remnant floodplains changing riparian biodiversity (Johnson 2002). This part of the Colorado River was most changed by the construction of a dam near the confluence of the Colorado and Concho rivers, which became Lake O. H. Ivie (Crisp 2012; Williams 2016). The primary disturbance and stresses influencing bottomlands in semi-arid regions have been associated with river flow where flooding resets the successional cycle (Shafroth et al. 2002; Hardy and Davis 2015) and provides inundation critical for wetland adapted plants (Dawson et al. 2017). In a study that examined hydrology, green ash and Texas persimmon were found to have the highest percent survival in drier stretches of the river (Kolka et al. 1998). At our site, shrubs like honey mesquite, Texas persimmon, and western soapberry are regenerating more often than other woody species (Table 2). Dawson et al. (2017) found native soil propagule banks to be intact along rivers, which after appropriate inundation can produce diverse mixtures of potential natural climax species and Alldredge and Moore (2014) found that changes in flood magnitude limited occurrence of wetland species. Wetlands are defined by having species with greater than 50% obligate or facultative wetland categories (Alldredge and Moore 2014) and government agencies have expanded their wetland definition to include bottomland hardwood forests (Kellison and Young 1997). The Colorado River bottomland forest was not dominated by wetland species. Alldredge and Moore (2014) reported this to be true of the Sabine River in East Texas as well. Riparian areas should contain a mix of obligate wetland, facultative wetland, and facultative species depending on water availability and it is important that riparian areas have species from the facultative group to provide stability due to hydrological change (Asher et al. 2015). This stretch of the Colorado River was mostly facultative upland and upland species with the only facultative wetland species present in the Colorado River bottomland being black willow and spiny aster.

Jones-Lewey (2016) listed indicators of riparian health, which include an active floodplain, energy dissipation during floods, new plant colonization, stabilizing vegetation, age diversity, species diversity, plant vigor, water storage, and establishment of equilibrium between erosion and deposition. At our study site, the Colorado River rarely reaches the bottomland due to upstream damming. When it does flood, there is such an increased flow that energy is often not dissipated by vegetation, which results in disequilibrium in erosion and deposition (Nelson personal observation). Therefore, we found areas with poor plant colonization in the near-riparian (Nelson unpublished data) but also due to high-levels of herbivory. There is stabilizing vegetation in the bottomland forest but it is disproportionately upland plants with wetland plants being rare. Jones-Lewey (2016), pointed out that the lack of wetland plants likely indicates poor water storage capacity for the bottomland. Based on the evaluation system of Jones-Lewy (2016), this portion of the Texas Colorado River was in an at-risk condition and will require management to restore it to a highly functional condition.

Diversity at this site on the Colorado River is different from other studies. The nearest location to the Colorado River that has examined diversity is the Bosque River in Erath County, which is about 120 km northeast of the Timberlake Ranch study site. It had higher diversity in every category except richness of herbaceous species and seedlings when compared to the Colorado River. There were few seedlings in the Colorado River bottomland, so much of the richness was due to herbaceous species, which may have been higher due to disturbance by herbivores and altered flood regimes. These relatively high levels of herbivory were lacking in the Bosque River bottomland (Rosiere et al. 2013). Nixon et al. (1990) reported mean Shannon-Weiner diversity for trees (>1 cm diameter) from four sites in the upper basin of the Trinity River, which was dominated by cedar elm, sugarberry, and green ash, as 2.5. This was higher diversity than that of the Bosque (2.2) and the Colorado (1.5) rivers. Nixon et al. (1990) examined woody plant communities within the Trinity River basin and reported that the less moist, western communities had lower mean diversity. Our data from the Colorado River supported this trend. It is also possible that the degradation of the Colorado River in terms of reduced hydrology and high herbivory contributed to its low diversity values in trees (> 1.0 cm in diameter) but lower levels of precipitation in the west might have a greater effect as hypothesized by Nixon (1990).

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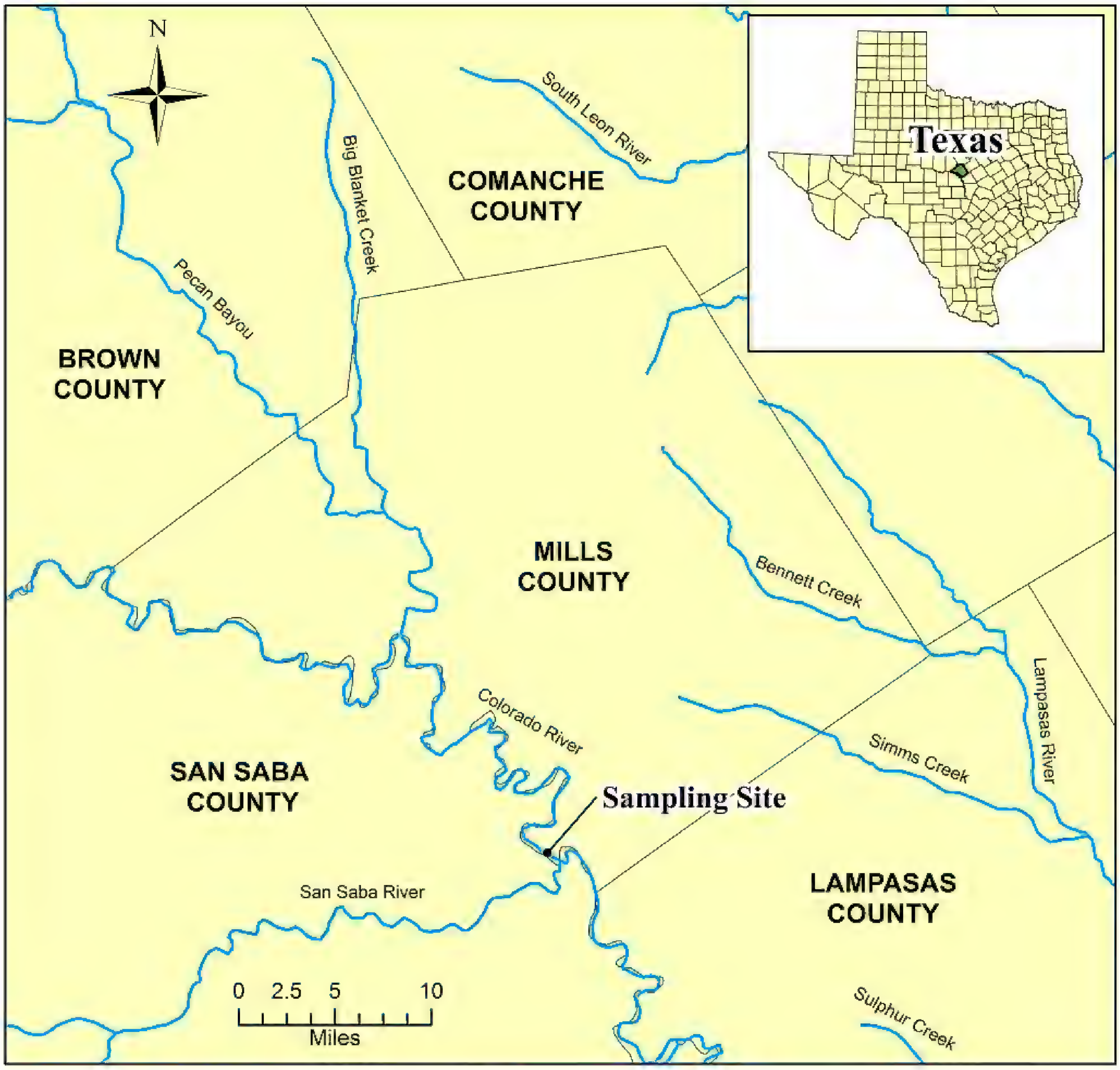

Figure 1. Map showing sampling site along the Colorado River in Mills County, Texas. Inset shows the location of the county in Texas.

Table 1-- Density, dominance, and relative importance values for woody vegetation greater than one centimeter diameter breast height of mixed hardwood bottomland forest of Colorado River, Texas. Wetland classification (Lichvar et al., 2016) is provided after the scientific name.

Common name (Scientific name) Wetland Classification	Density (plants/ha)	Dominance (m^2/ha)	Importance Value (%)
American elm (*Ulmus americana*) Facultative	24.0	37.1	3.9
Black willow (*Salix nigra*) Faculative Wetland	1.3	0.1	0.1
Cedar elm (*Ulmus crassifolia*) Facultative	222.7	1036.8	70.7
Chittamwood (*Sideroxylon languinosum* subsp. *oblongifolium* Facultative upland	4.0	0.1	0.3
Cottonwood (*Populus deltoides*) Facultative	4.0	8.9	1.2
Flame-leaf sumac (*Rhus lanceolata*) Upland	1.3	< 0.1	0.1
Green ash (*Fraxinus pennsylvanica*) Facultative	45.3	204.9	14.1
Honey mesquite (*Prosopis glandulosa*) Facultative upland	1.3	< 0.1	0.1
Pecan (*Carya illinoinensis*) Facultative	2.7	<0.1	0.3
Prickly-pear cactus (*Opuntia engelmannii* var. *lindheimeri*) Upland	2.7	16.8	1.1
Sugarberry (*Celtis laevigata* var. *laevigata*) Facultative	25.3	14.9	2.9
Texas persimmon (*Diospyros texana*) Upland	14.7	0.1	1.2
Western soapberry (*Sapindus saponaria*) Facultative upland	46.7	10.8	4.5
Total	396.0	1,319.7	101.5

Table 2--Species composition as determined by step-point method herbaceous and woody plants (below 1 cm diameter) of mixed hardwood bottomland forest of Colorado River, Texas. An asterisk indicates an introduced species. Vegetation categories and their totals are in italics. Wetland classification ((Lichvar et al., 2016) is provided after the scientific name.

Common name (Scientific name)	No. hits (%)
Grasses	
*Bermudagrass (*Cynodon dactylon*) Facultative upland	191 (5.0)
Big-top love grass (*Eragrostis hirsuta*) Facultative upland	2 (< 0.1)
Canada wildrye (*Elymus canadensis*) Facultative upland	783 (20.6)
Hall's panic (*Panicum hallii* var. *hallii)* Facultative upland	28 (0.7)
*Japanese brome (*Bromus japonicus*) Upland	1 (< 0.1)
*Klein grass (*Panicum coloratum*) Facultative	1 (< 0.1)
Nimblewill (*Muhlenbergia schreberi*) Facultative upland	4 (0.1)
Purpletop (*Tridens flavus*) Upland	2 (<0.1)
Southwestern bristlegrass (*Setaria scheelei*) Upland	91 (2.4)
*Stink grass (*Eragrostis cilianensis*) Facultative upland	4 (0.1)
Texas wintergrass (*Nasella leuchotricha*) Upland	577 (15.2)
Total Grasses	1684 (44.3)
Grasslike	
Gotthilf Muhlenenberg's caric-sedge (*Carex muhlenbergii* var. *muhlenbergii*) Upland	131 (3.4)
Unknown caric sedge (*Carex* sp.)	94 (2.5)
Unknown flat sedge (*Cyperus* sp.)	5 (0.1)
Total Grasslike	230 (6.1)
Forbs	
Betony noseburn (*Tragia betonicifolia*) Upland	67 (1.8)
Cowpen daisy (*Verbesina encelioides*) Facultative	62 (1.6)
Creeping ladies'-sorrel (*Oxalis corniculata*) Facultative	19 (0.5)
Frostweed (*Verbesina virginica*) Facultative upland	7 (0.2)
*Horehound (*Marrubium vulgare*) Facultative upland	60 (1.6)
Indian mallow (*Abutilon fruticosum*) Upland	5 (0.1)
Pigeonberry (*Rivina humilis*) Facultative	72 (1.9)
Silver-leaf nightshade (*Solanum elaeagnifolium*) Upland	25 (0.7)
Spiny-aster (*Chloracantha spinosa*) Facultative wetland	51 (1.3)
Texas croton (*Croton texensis*) Upland	7 (0.2)
Unknown mint	1 (< 0.1)
Virginia copperleaf (*Acalypha virginica*) Facultative upland	29 (0.8)
Total Forbs	405 (10.7)
Shrubs, lianas, and small trees	
Cedar elm (*Ulmus crassifolia*) Facultative	2 (< 0.1)
Flame-leaf sumac (*Rhus copallinum* var. *latifolia* Upland	1 (< 0.1)
Honey mesquite (*Prosopis glandulosa*) Facultative upland	28 (0.7)
Juniper (*Juniperus* sp.)	1 (< 0.1)
Rusty blackhaw (*Viburnum rufidulum*) Facultative upland	1 (< 0.1)
Saw Greenbriar (*Smilax bona-nox*) Facultative upland	57 (1.5)
Sugarberry (*Celtis laevigata* var. *laevigata*) Facultative	4 (0.1)
Tasajillo (*Opuntia leptocaulis*) Upland	1 (< 0.1)
Texas persimmon (*Diospyros texana*) Upland	15 (0.4)
Texas prickly-pear (*Opuntia engelmannii* var. *lindheimeri* Upland	1 (< 0.1)

Unknown shrub	1 (< 0.1)
Table 2 (cont.) Western soapberry (*Sapindus saponaria*) Facultative	16 (0.4)
Total shrubs and small trees	128 (3.4)
Bare ground	1353 (35.6)
Total Hits	3800 (100.1)

Table 3. Number of samples and percentages of beaver damage in the bottomland (*n*=85) of the Colorado River at Timberlake Ranch.

Beaver damaged woody vegetation	Bottomland (%)
Cedar elm	57 (67.1)
Texas persimmon	13 (15.3)
Sugarberry	12 (14.1)
Cottonwood	2 (2.4)
Green ash	1 (1.2)

Table 4. Richness, Evenness, and Shannon Diversity in the Texas Colorado (TXCO) River Bottomland to that of the Bosque River (Roseiere et al., 2013).

	TXCO River	Bosque River
Woody Richness > 1.0 cm	13	17
Herbaceous & seedling Richness < 1.0 cm	40	30
Woody Evenness > 1.0 cm	0.59	0.77
Herbaceous & seedling Evenness < 1.0 cm	0.66	0.66
Woody Shannon Diversity > 1.0 cm	1.51	2.18
Herbaceous & seedling Shannon Diversity < 1.0 cm	2.42	2.26

Preliminary examination of hybridization and introgression between *Juniperus flaccida* and *J. poblana*: nrDNA and cpDNA sequence data

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ABSTRACT

Analyses of nrDNA and cpDNA (petN-psbM, trnSG, trnDT, trnLF) from *J. flaccida, J. poblana, J. p.* var. *decurrens*, and putative hybrids revealed some populations of pure *J. flaccida*, and a few areas of pure trees of *J. poblana*. However, most *J. poblana* or putative hybrids in the eastern part the range were found to be introgressed by chloroplast capture by *J. flaccida*. A few putative hybrids (*J. flaccida* × *J. poblana)* were confirmed by the presence of heterozygous specific indicator bases at nrDNA site 768. Introgression of *J. flaccida* into *J. poblana* appears to be widespread in eastern Mexico. *Phytologia 100(2): 145-152 (Jun 22, 2018).* ISSN 030319430.

KEY WORDS: *J. flaccida, J. poblana*, Cupressaceae, hybridization, nrDNA, trnSG DNA.

Juniperus flaccida Schltdl. and *J. poblana* (Martínez) R.P. Adams are closely related species with large, multi-seeded cones and weeping (flaccid) foliage that make them difficult to differentiate morphologically (Adams 2014). In some areas they appear to grow together and appear to hybridize.

In January 2018 Allen Coombes and Lucio Caamaño O. visited the type locality of *J. poblana* as well as the municipio of Cuautinchán in order to investigate the extent of distribution of *J. poblana.* It was observed that typical *J. poblana* appeared to be restricted to north-facing slopes close to the type locality while further south and at lower altitudes the junipers showed a great deal of variation with some appearing closer to *J. flaccida.* This work is a result of a follow-up visit to the area with Socorro González.

Recently, Adams et al. (2018) re-examined *Juniperus flaccida* and *J. poblana*. They reported that analysis of nrDNA, petN-psbM, trnS-trnG, trnD-trnT, and trnF-trnL revealed that *J. flaccida* is diverse, but distinct from *J. poblana* from Amozoc and *J. poblana* from Nayarit by at least 10 Mutational Events (MEs, SNPs + indels). Divergent *J. poblana* from Oaxaca is 2 MEs distant from the Nayarit trees. The *J. poblana* samples from the type locality (Amozoc, Pue.) vary by only 1 ME among samples. Leaf oils, on the other hand, show that the Nayarit juniper is quite differentiated from other *J. poblana.*

This paper is a preliminary report on possible hybridization and introgression between *J. flaccida* and *J. poblana* utilizing nrDNA and cp DNA (trnS-trnG) sequence data.

MATERIALS AND METHODS

Plant material and populations studied:

Putative *J. flaccida* - *J. poblana* hybrids:

1.4 km al S de San José Laguna, Mpio. Amozoc, Edo. Pue. 19° 01' 15" N, 98° 01' 57.8" W, 2317 m, 11 Sep 2017. Remanente de bosque de *Juniperus*. Arbol de 2 - 5 m de altura, Coll. Lucio Caamaño 11265, Allen Coombes, S. González; Lab Acc. *Robert P. Adams No. 15296*

1.6 km al S de Amozoc por carretera a Santa Cruz, Mpio. Amozoc, Edo. Pue. 19° 01' 42.4" N, 98° 05' 17.5" W, 2335 m, 11 Sep 2017. Bosque de encino, arbol de 4 m de altura. Coll. *Lucio Caamaño 11276-a, Allen Coombes, S. González*; Lab Acc. *Robert P. Adams 15297*

1.6 km al S de Amozoc por carretera a Santa Cruz, Mpio. Amozoc, Edo. Pue. 19° 01' 42.4" N, 98° 05' 17.5" W, 2335m, 11 Sep 2017. Bosque de encino, arbol de 5 - 6 m de altura, Coll. *Lucio Caamaño 11277, Allen Coombes, S. González*; Lab Acc. *Robert P. Adams 15298*

2.4 km por camino de terraceria Av. Gonzalo Bautista Pte. al W de Cuautinchán, Mpio. Cuautinchán, Edo. Pue. 18° 57' 29.3' N, 98° 1' 54' W, 2170 m, 11 Sep 2017. Vegetación secundaria a orilla de río, arbol de 8 m de altura, Coll. *Lucio Caamaño 11286, Allen Coombes, S. González*; Lab Acc. *R Adams 15299*

Al W de Alpatlahuac, Mpio. Cuautinchán, Edo. Puebla, 18° 55' 50" N, 98° 2' 24.1" W, 2115 m, 11 Sep 2017. Vegetación secundaria asociada a *Juniperus*, arbol de 8 - 9 m de altura, Coll. *Lucio Caamaño 11287, Allen Coombes, S. González,* Lab Acc. *R Adams 15300*

Al W de Alpatlahuac, Mpio. Cuautinchán, Edo. Puebla, 18° 55' 50" N, 98° 2' 24.1" W, 2115m, 11 Sep 2017. Vegetación secundaria asociada a *Juniperus*, arbol de 8 m de altura, Coll. *Lucio Caamaño 11290, Allen Coombes, S. González,* Lab Acc. *R Adams 15301*

Reference specific samples:

J. flaccida

J. flaccida, Adams 6892-6896, 24° 41' 54"N, 100° 04' 59" E, 5800 ft., 23 km E of San Roberto Junction on Mex. 58, Nuevo Leon, Mexico;

J. flaccida, Reserva Ecologica Municipal de Sierra y Cañon de Jimulco, 25° 07' 38" N, 103° 16' 15" W, 2118 m, 17 Jan 2017, Torreon, Coahuila, Mexico, Coll. *Manuel Rodríguez Munoz et al. #1,2,3,4,5,* Lab Acc. *Adams 15203 - 15207*;

J. flaccida, Sierra del Rosario, Durango, Mexico, 25° 38' 44" N, 103° 54' 40" W, 2700 m, with *Yucca carnerosana*, *Agave gentryi*, and *Quercus* scrub, 27 Apr 2008, Coll. *M. S. Gonzalez-Elizondo et al. 7375a, b*, Lab Acc. *Adams 14616 – 14617*;

J. poblana

J. poblana, scattered, at Km 62, 62 km S of Oaxaca on Mex 190. 16° 41' 36" N, 96° 19' 41" W, 1710m, 19 Dec 1991, Oaxaca, Mexico, Coll: *Robert P. Adams 6868-6872*

J. poblana, uncommon young trees (saplings) 2 m, in oak woodland dominated by *Quercus resinosa*, Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz, Km 86.8; S of bridge of arroyo del Fraile, E of El Maguey, 22° 10' 08" N, 104° 43' 51" W, 1150 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8381 with L. López, A. Torres Soto;* Lab Acc. *Robert P. Adams 14896*

J. poblana, large, single stemmed trees, foliage long and pendulous, abundant trees, up to 25 m high, on strongly rocky slope, forest of *Juniperus-Clusia* with elements of mesophytic forest (*Magnolia*) and tropical forest (*Bursera*, *Opuntia*, *Pilosocereus purpusii*) as well as *Agave attenuata* and *Yucca jaliscensis,* Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz; NE of El

Maguey, 22° 07'40" N, 104° 47' 47" W, 1430 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8379a,b,c,d, with L. López, A. Torres Soto;* Lab Acc. *Robert P. Adams14897-14900,*

J. poblana, scattered in a *J. poblana* - oak forest. Amozoc de Mota, just s of town. lvs for spec, oil, DNA. 19° 01' N, 98° 01' W., 2300 m. Date 16 Dec 2016. Mpio. Amozoc, Type locality of *J. poblana*. Puebla, Mexico. Coll. *L. Caamaño* and *Allen Coombes 10172,10173,10174,10180,10181*. 2344-2358 m, 16 Dec 2016. Det. Socorro Gonzalez, Lab Acc. *Robert P. Adams 15208 - 15212.*

J. poblana var. *decurrens,* scattered

J. poblana var. *decurrens* R. P. Adams, small tree, 5m tall, with strong central axis, foliage very, very, weeping, female, common, about 2 km s of Valle de Topia. All leaves decurrent, and prickly. Not juvenile leaves. 25° 14' 11" N, 106° 26' 55.7" W, 1818 m, 30 June 30, 2009, Durango, Mexico. Coll. *Robert P. Adams 11926* with *Socorro and Martha Gonzalez.*

Voucher specimens are deposited at BAYLU, CIIDIR and HUAP when applicable.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 μM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

Sequencing nrDNA revealed that *J. flaccida* and *J. poblana* growing in uniform populations (assumed pure, as they are isolated from other areas, and their nrDNA is not polymorphic at any base sites) differed consistently by only 1 SNP at site 768. This SNP is G in *J. flaccida*, and A in *J. poblana* (Table 1). Comparison of sequencing for the reference populations of *J. flaccida* and *J. poblana* reveal few informative SNPs: trnSG 1 SNP (site 96), 1 indel; petN-psbM, 0 SNPs; trnLF: 1 SNP, 1 indel; trnDT: 2 SNPs, 1 indel. Because the classifications based on the cp DNA were consistent, only trnSG, site 96, was utilized to survey additional samples.

All the samples from NL (Nuevo Leon, 23 km east of San Roberto Junction) and from near Torreon, Coahuila were found to be uniform by having clean (homozygous) peaks of *J. flaccida* nrDNA (ITS) and also having *J. flaccida* cp DNA (trnSG). However, 'pure' *J. poblana* was found in the Nayarit population, 2 trees from Amozoc (15208, 15209), and 2 trees of *J. poblana* var. *decurrens* (11927, 11928, Table 1). Other *J. poblana* trees from Amozoc and Oaxaca had clean, *J. flaccida* nrDNA, but *J. poblana* cp DNA, suggesting chloroplast capture by hybridization and introgression (Table 1).

Several putative hybrids were sampled (Table 1). Sample 15296, Amozoc, had heterozygous nrDNA (G/A ~2:1), with *J. poblana* cpDNA. Recently, the inheritance of nrDNA polymorphisms has been reported (Adams and Matsumoto, 2016, Adams et al. 2016) in two Cupressaceae genera. In cultivars of *Cryptomeria japonica*, Adams and Matsumoto (2016) found that 'Haara' and 'Kumo' differ at three sites in their nrDNA. However, 'Kumo' was polymorphic at each of the three sites (indicating that 'Kumo' was a cross of 'Haara' with an unknown plant). Thus, the seven artificial crosses between 'Haara' and 'Kumo' are actually backcrosses to 'Haara'. They reported that three of the backcrosses were polymorphic at the three nrDNA sites, but four (of the seven crosses) were monomorphic (homozygous) at the three nrDNA sites! Thus, it appears that in this instance, some backcrosses may rather quickly eliminate heterozygous sites in favor of the recurring parent.

Adams et al. (2016) analyzed crosses between *Hesperocyparis* (= *Cupressus*) *arizonica* and *H. macrocarpa* that included the parents and 18 artificial hybrids. In this case, 8 nrDNA sites differed between *H. arizonica* and *H. macrocarpa*, and all of these sites were homozygous in each parent. Adams et al. (2016) reported the peak ratios in the sequencing chromatograms were not always 1:1 as expected, but varied from 1:1 to 3:1 (3:1 being more like *H. arizonica* nrDNA). They concluded that due to concerted evolution, analysis of heterozygous sites in nrDNA may underestimate the degree of hybridization and introgression. Nevertheless, all of the 18 artificial hybrids were polymorphic at each of the 8 sites, and this clearly revealed that they were hybrids (although, from the data, one might presume some plants were backcrosses or introgressants). Only 3 of the 18 hybrids had nearly 1:1 ratios of the bases of the parents at all 8 sites. This paper (Adams et al. 2016) is very relevant to the present (and future) studies in *Juniperus*, as *Hesperocyparis* is very closely related to *Cupressus* and *Juniperus* (Adams, Bartel and Price, 2009; Terry et al. 2016), so it seems likely that the mode of inheritance in *Juniperus* nrDNA is similar to that found in *Hesperocyparis*.

Examination of the six putative hybrids (ex per field observations) shows (Table 1) that two (15296, 15300) were heterozygous at site 768 indicating they are hybrids (or backcrosses). The remaining four samples, each had *J. flaccida* nrDNA and *J. poblana* cpDNA, implying ancient chloroplast capture by introgression.

To visualize the distribution of putative hybridization and introgression, samples were mapped. Their nrDNA and cpDNA were denoted by icons (Fig. 1). The northernmost populations of *J. flaccida* appear to be 'genetically pure' (for nrDNA and cpDNA) and they are geographically isolated from *J. poblana*. The only 'genetically pure' *J. poblana* plants were in Nayarit, two plants from Amozoc, and two plants of *J. poblana* var. *decurrens*.

The easternmost distributed *J. poblana* - hybrids generally display (Fig. 1) a pattern of chloroplast capture with introgression from *J. flaccida* (Fig. 1). Only two 'genetically pure' *J. poblana* plants were found in the eastern region (near Amozoc).

Finally, it is interesting to analyze the volatile oils of samples of *J. poblana* var. *decurrens*: Adams 11926 (flac, pob), Adams 11927 (pob, pob), Adams 11928 (pob, pob). The leaf volatile oils differ little between *J. flaccida* and *J. poblana* (Adams and Zanoni, 2015; Adams and Schwarzbach, 2015; Adams, Gonzalez-Elizondo and Gonzalez-Elizondo, 2017b). However, *J. flaccida* and *J. poblana* differ in their concentrations of linalool, δ-2-carene, δ-3-carene, naphthalene, methyl chavicol, cubebol and (E)-nerolidol. In 11926, the concentrations of linalool, δ-3-carene, naphthalene, and cubebol are transgressive (i.e., Table 2, outside the range of the putative parents). For δ-2-carene and (E)-nerolidol, 11926 is very similar to *J. flaccida*. For methyl chavicol, it is very similar to *J. poblana*.

Interestingly, analyses of these seven compounds for plants 11927 and 11928 showed some of the same transgressive patterns (Table 2). This is not surprising, because studies on the inheritance of terpenoids in *Cryptomeria japonica* (Adams and Tsumura, 2012) and *Pseudotsuga menziesii* (Adams and Stoehr, 2013) artificial hybrids revealed that many compounds are transgressive in the hybrids and some are intermediate in concentration between the parents' values.

Classification of the seven oil constituents by parent types (Table 4) revealed that 11926 and 11928 have the same patterns except for δ-2-carene. In contrast, 11927 displays a different patterns (from that of 11926, 11928) for δ-3-carene, cubebol and (E)-nerolidol. This seems to indicate that there is more genetic mixing in the *J. poblana* var. *decurrens* population than as revealed in the nrDNA data.

Although we have sampled from much of the range of *J. poblana* (Figs.1, 2, 3), there is a large portion of the range of *J. flaccida* that was not been sampled in this study (Figs. 1,2,3). Additional sampling is needed from central Mexico and more extensive sampling is needed in the *J. poblana* var. *decurrens* population, as well as in the Amozoc - Cuautinchán area.

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Table 1. Analyses of putative hybrids of *J. flaccida* x *J. poblana*, utilizing nrDNA and trnSG sequences. *J. flaccida* and *J. poblana* nrDNA differs at only 1 SNP at site768 and their trnS-G differs by only 1 SNP at site 96.

Reference Samples Collected as:	nrDNA, site 768/ classification (A/G)	trnS-G, site 96 / classification	combined classification
6892 flaccida, San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6893 flaccida, San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6894 flaccida, San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6895 flaccida , San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6896 flaccida , San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
15203 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15204 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15205 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15206 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15207 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
14616 flaccida, S. del Rosario, Dur.	G clean, flaccida	A/ flaccida	flaccida
14617 flaccida, S. del Rosario, Dur.	G clean, flaccida	A/ flaccida	flaccida
14896 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14897 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14898 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14899 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14900 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
15208 poblana, Amozoc	A clean, poblana	G/ poblana	poblana
15209 poblana, Amozoc	A clean, poblana	G/ poblana	poblana
15210 poblana, Amozoc	G clean, flaccida	G/ poblana	introgressant?
15211 poblana, Amozoc	G clean, flaccida	G/ poblana	introgressant?
15212 poblana, Amozoc	G clean, flaccida	G/ poblana	introgressant?
6868 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6869 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6870 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6871 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6872 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
11926 poblana v. *decurrens*	G clean, flaccida	G/ poblana	introgressant?
11927 poblana v. *decurrens*	A clean/ poblana	G/ poblana	poblana
11928 poblana v. *decurrens*	A clean/ poblana	G/ poblana	poblana
Collected as putative hybrids:			
15296, Amozoc	G/A (2:1)	G/ poblana	hybrid/backcross
15297, Amozoc	G, flaccida	G/ poblana	introgressant?
15298, Amozoc	G, flaccida	G/ poblana	introgressant?
15299, Cuautinchán	G, flaccida	G/ poblana	introgressant?
15300, Cuautinchán	G/A (4:1)	G/ poblana	backcross
15301, Cuautinchán	G, flaccida	G/ poblana	introgressant?

Table 2. Comparison of selected terpenes in *J. flaccida*, *J. poblana*, and individuals of *J. poblana* var. *decurrens* (* = 11926 (ITS flacc., cp pob), # = 11927 (ITS pob, cp pob), $ = 11928 (ITS pob, cp pob).

Cpd.	*J. flaccida* (avg). *J. poblana* (avg)
linalool	2.9F..1.6P.........1.0#....................0.3$.....0.1*
δ-2-carene	0.0F....0.05*...1.6$ 1.8P 1.9#
δ-3-carene	0.0F 0.1#...1.4P................3.7*..................6.9$
naphthalene	0.0F..0.05P..0.3$........0.3#....................0.5*
methyl chavicol	0.0F...0.5$..0.6*..0.7P..0.8#
cubebol	0.0F..0.0P 0.1#........0..4$....................1.1*
(E)-nerolidol	0.0F 0.1# 0.3$...............1.2#..........................2.5P

Table 3. Classification of *J. p*oblana var. *decurrens* individuals by 7 distinctive components. Parent types: F = flaccida, P = poblana; I = intermediate. >P = cpd > conc. in poblana; >F = cpd > conc. in flaccida.

Component conc. like: F, *flaccida*, P, *poblana* or I, intermediate, <, >.			
Cpd.	11926 *	11927 #	11928 $
linalool	<P	<P	<P
δ-2-carene	F	P	P
δ-3-carene	>P	F	>P
naphthalene	>F>P	>F>P	>F>P
methyl chavicol	P	P	P
cubebol	>F>P	F,P	>F>P
(E)-nerolidol	F	intermediate	F

Table 3. Classification of components by parent types: F = *flaccida*, P = *poblana*; I = intermediate. >P = cpd > conc. in *poblana*; >F = cpd > conc. in *flaccida*.

J. p. var. *decurrens*	linalool	δ-2-carene	δ-3-carene	naphthalene	methyl chavicol	cubebol	(E)-nerolidol
11926 *	<P	F	>P	>F>P	P	>F>P	F
11927 #	<P	P	F	>F>P	P	F,P	intermediate
11928 $	<P	P	>P	>F>P	P	>F>P	F

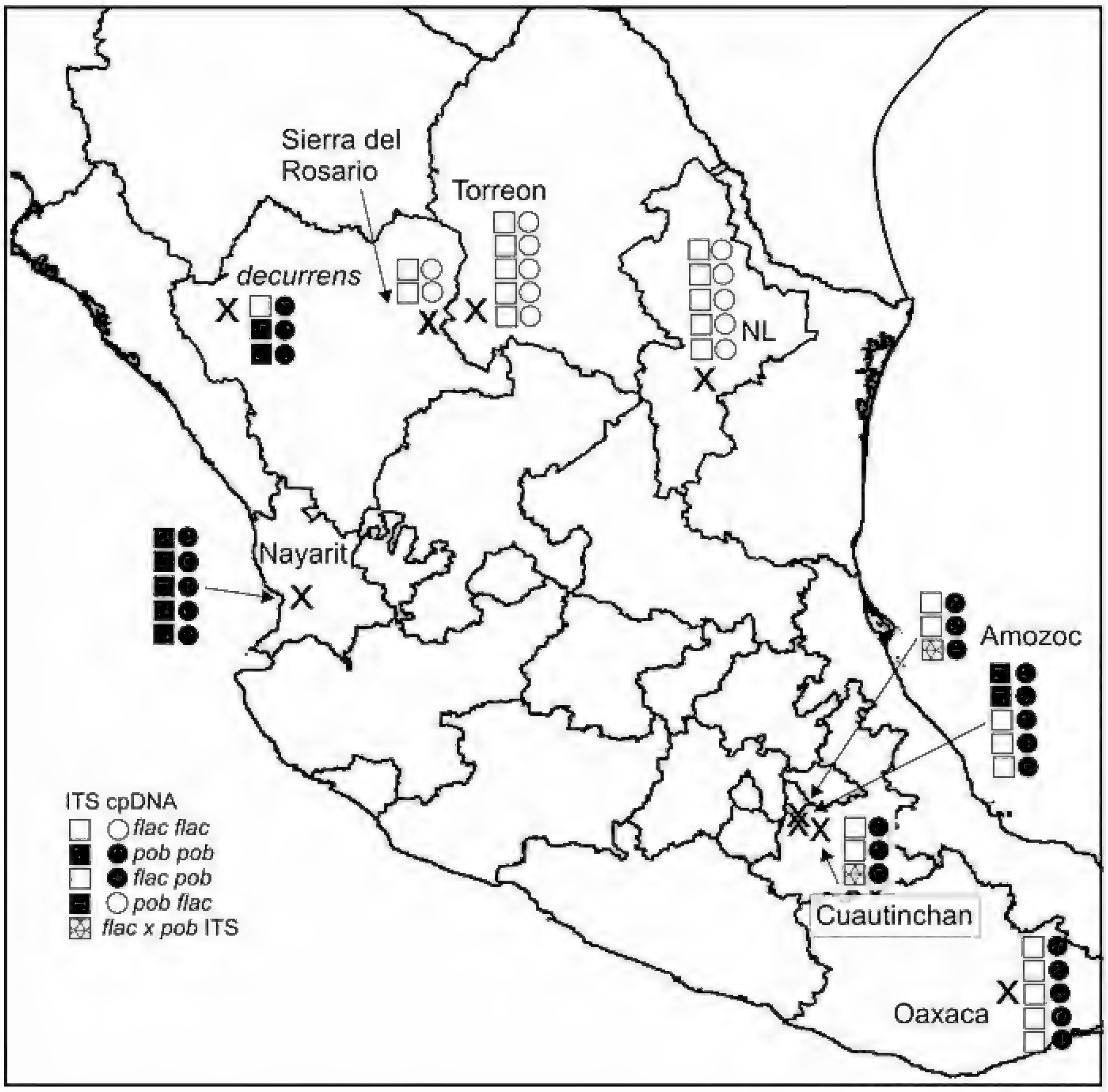
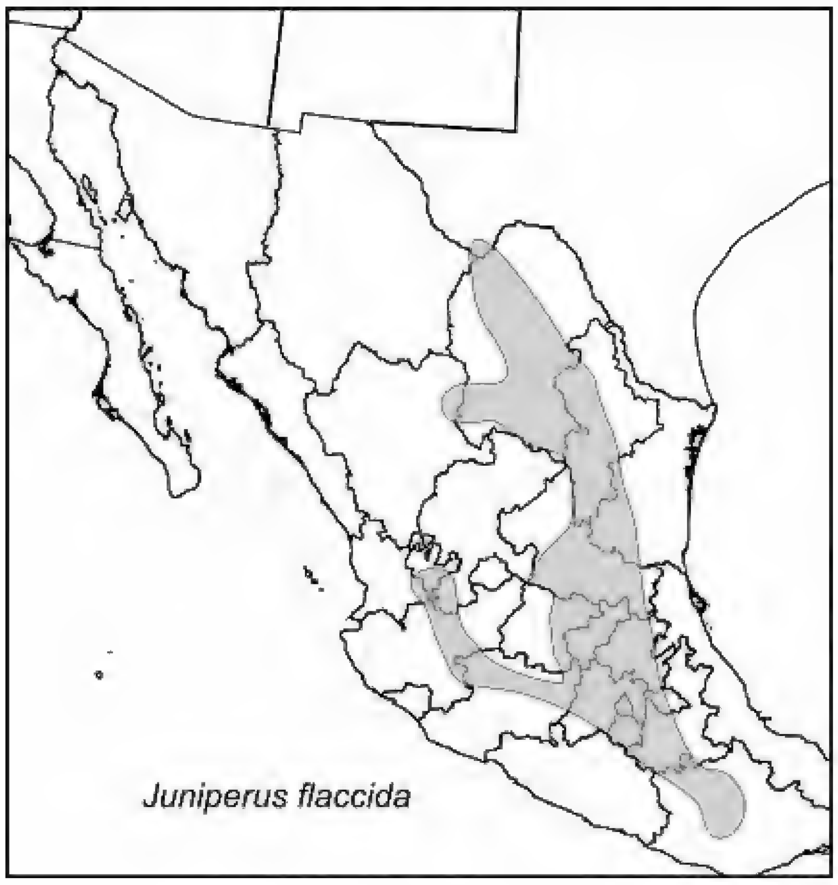
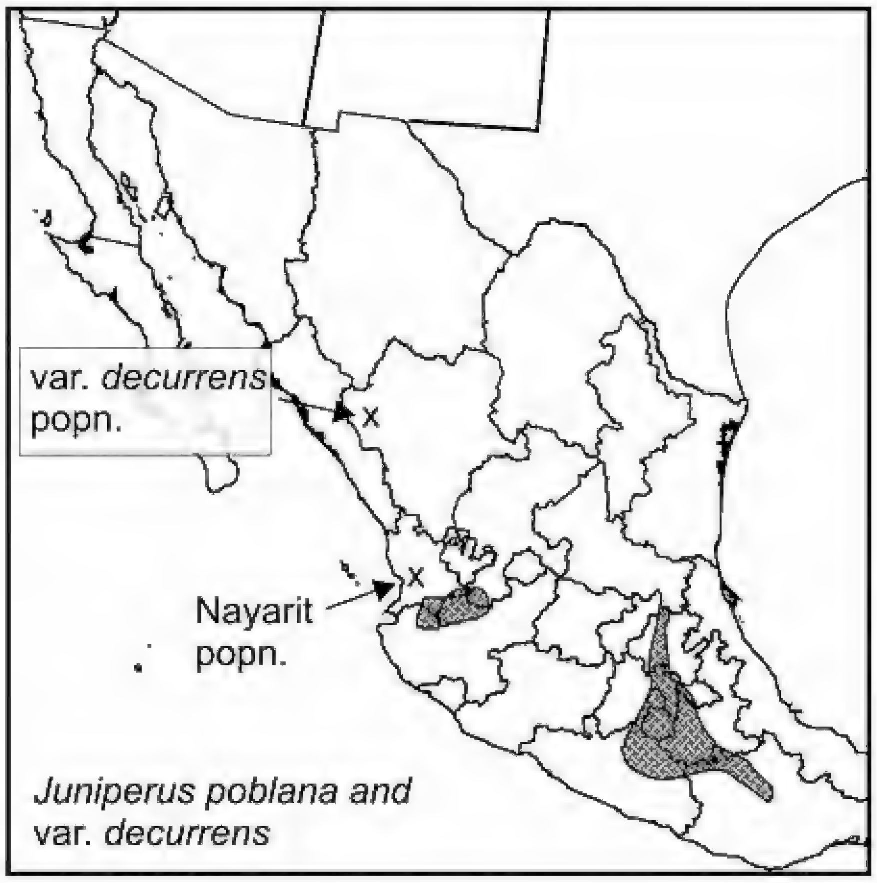


Figure 1. Distribution of ITS (nrDNA), cpDNA classifications of *J. flaccida* and *J. poblana*, and putative hybrids. See text for discussion.





Figures 2 (left) and 3 (right). Distributions of *J. flaccida* and *J. poblana* (modified from Adams, 2014).

Geographic variation in hexane extractable hydrocarbons in natural populations of *Helianthus annuus* (Asteraceae, Sunflowers) II.

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ABSTRACT

Populations of *Helianthus annuus* L., ranging from eastern Oklahoma to North Dakota, to coastal southern California were sampled and the yields of total hydrocarbons (HC) from leaves determined. The highest yielding populations were in the Texas Panhandle (6.0 - 7.99%) and the lowest yields were in Camp Verde, AZ, NM mountains, Bozeman, MT, and ND - MN. Medium-high yields were found in northern UT and southern ID. Three populations near Waco, TX had large yield differences ranging from 4.9 to 6.2%, but a fourth population had a low 3.6%. Some native populations were contaminated by germplasm from cultivated sunflowers and these populations had very low yields (2.6 - 3.6%). Population variability in HC yields varied geographically and also between nearby populations, suggesting the micro-habitat environments are important as well as limited genetic population size. Published on-line www.phytologia.org *Phytologia 100(2): 153-160 (Jun 22, 2018).* ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, geographic variation in leaf hydrocarbon yields.

Adams and Seiler (1984) surveyed 39 taxa of sunflowers for their cyclohexane (hydrocarbon) and methanol (resins) concentrations. The highest cyclohexane (bio-crude) yielding taxa were *H. agrestis*, an annual, Bradenton, FL (7.38%) and *H. annuus*, Winton, OK (7.09%). Adams et al. (1986) screened 614 taxa from the western US for their hydrocarbon (hexane soluble) and resin (methanol soluble) yields. They reported 2 plants of *H. annuus* from Idaho with 8.71% and 9.39% hydrocarbon yields.

Seiler, Carr and Bagby (1991) reported on 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. The rubber was found to be of lower molecular weight than *Hevea* rubber, but still appeared to be useful as a plasticizing additive and for coatings the inside of pipes and containers.

Yields of natural rubber has recently been reported for *H. annuus* (Pearson et al. (2010a) that ranged from 0.9% to 1.7% rubber in cultivated sunflower cultivars (Pearson et al. 2010b, Fig. 4,).

Most recently, Adams et al. (2018) reported on geographical variation in natural rubber yields in *H. annuus*. They found considerable variation in yields of natural rubber with the highest yielding populations at Mill Creek, UT and in the Waco, TX area. These high yielding populations are in very different eco-systems with different climates and soils. The rubber concentrations in adjacent plants sometimes varied from none to 16 mg/g.

Recently, Adams et al. (2017a) reported on hydrocarbon yields (HC) from a large area from Oklahoma to southern California. This report is a continuation of that survey of HC in sunflowers as we focus on basic research on *H. annuus* (Adams and TeBeest, 2016; Adams, et al. 2016).

MATERIALS AND METHODS

Population locations - see Appendix I.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

RESULTS

The yields of hydrocarbons (HC) by population are given in Table 1. The highest yield (8.60%) was from Gruver, TX followed by Lake Tanglewood, TX (8.47%) in the Texas Panhandle. The lowest yield was from Woodward, OK (2.62%), Eagle Nest, NM (2.62%) and Clay county, MN (2.61%) followed by cultivated sunflowers (Oslo, TX)(3.20%). The Woodward population had smooth leaves as found in cultivated sunflowers. The plants and leaves were very large, although the heads were small. It appears that the Woodward population was a product of crosses between native and cultivated sunflowers and this resulted in the very low oil yield.

To visualize the variation in HC yields, the means were contour mapped (Fig. 1). Notice that the highest yields are in the Texas Panhandle. The west (EN, AZ) is region of low yields (Fig.1). A mosiac pattern of HC yields was found in sw Utah with low yield at KU (Kolob Canyon, 3.77%), but a high yield about 20 mi away at Leeds, UT (LU, 7.01%, Fig. 1). Another area of low yields is in North Dakota - Minnesota (GN, 3.23%, and CM, 2.1.6%, Fig. 1). These and the WO (Woodward, OK) are likely of hybrid origin between native and cultivated sunflowers. The southern Idaho - northern Utah area had medium - high yields. Of interest are the four populations near Waco, TX (MC, FC, LC, HC) that have 6.2, 5.3, 3.6, and 4.9% yields in a very small area. Adams et al. (2017b) examined the HC yields from naturally grown parents vs. green house grown progeny and reported much lower HC yields in greenhouse grown progeny compared to the parents growing under harsh environmental conditions. They concluded that up to half the HC production in nature is induced by insect damage and harsh environmental conditions. It may be that HC can be induced by chemicals sprayed on crops. It is very clear, from their greenhouse grown plants that high yields are not constitutive in *H. annuus*, but, rather, are induced by some factor not present in the greenhouse.

Adams et al. (2017a) found that the correlation between % yield and leaf weight as only r = 0.18 (highly significantly different from zero, df = 327). But the correlation accounted for only 3.24% (r^2) of the variance. They concluded that breeding for both increased % yields of HC and biomass seems feasible.

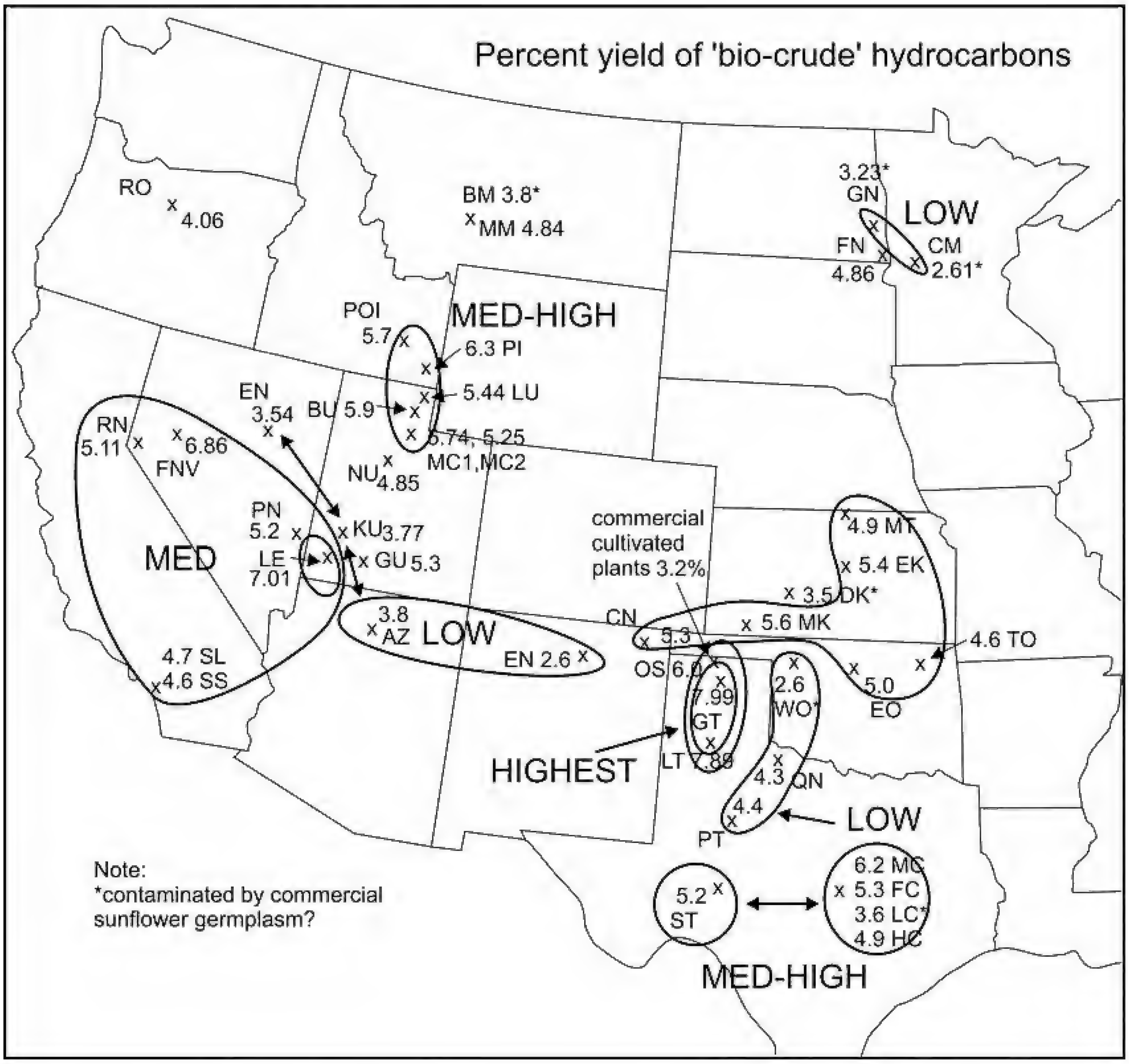

Figure 1. Geographic variation in % yields of HC by population. The asterisk (*) at the WO, LC, CM, GN, BM populations indicates that these population may be of hybrid origin between native and cultivated sunflowers.

The variability of yields by population is mapped in Figure 2. Population variability in HC yields varied geographically and also between adjacent populations, suggesting the micro-habitat environments are important as well as limited genetic population size. Some of the least variable populations were in North Dakota (FN, GN) and Minnesota (CM, Fig. 2). These populations appear to be introgressed from cultivated sunflowers, so that may explain the low variation.

Clearly the most unusual situation was the Waco, TX area where 4 nearby populations (MC, FC, LC, HC, Fig. 2) showed very small to large amounts of variation in their HC yields. MT (Montrose, KS) was from only 3 cultivated plants raised from seed, so its small variability may be just by chance.

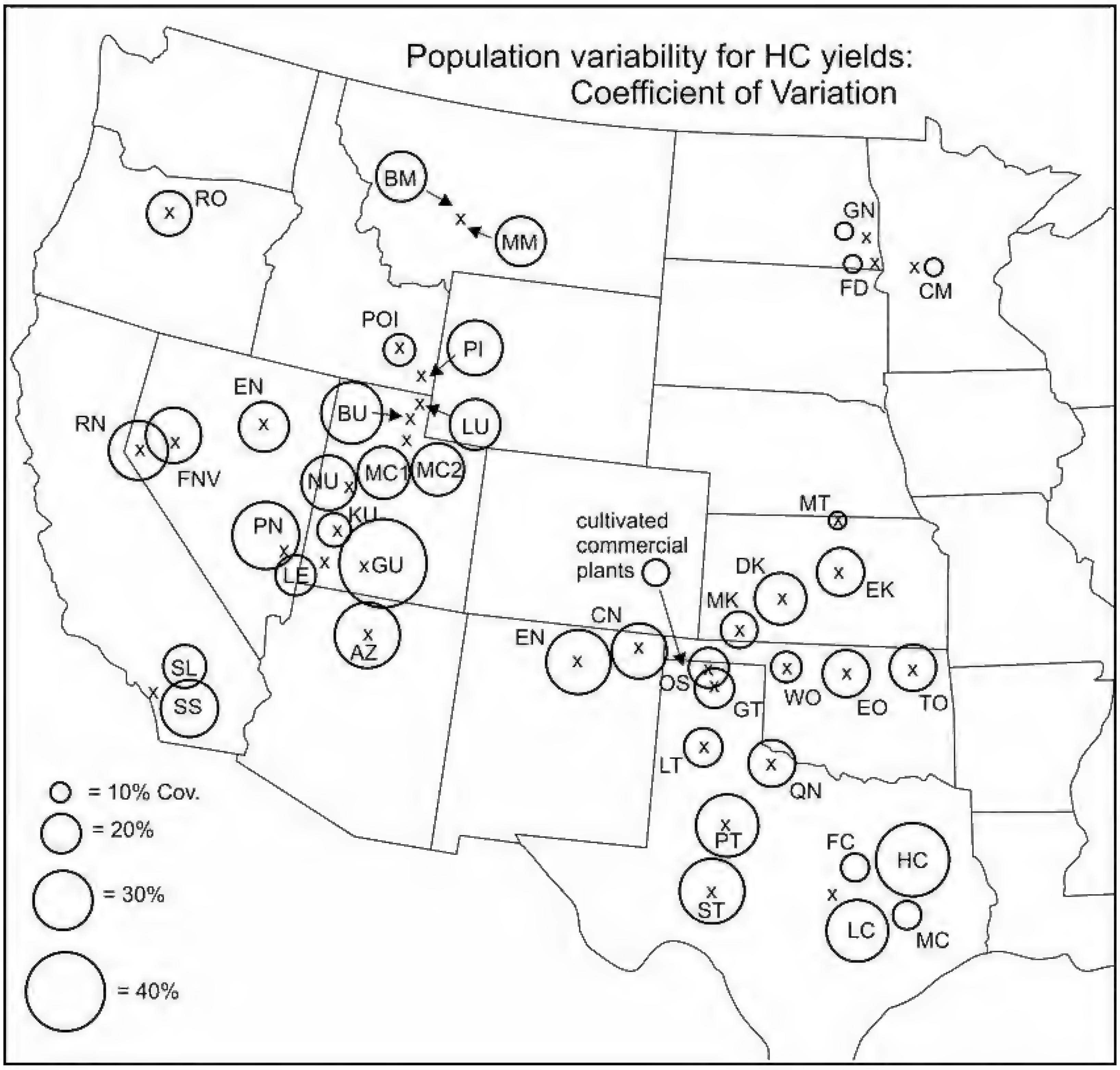

Figure 2. Population variability (coefficient of variation in HC yields) for the populations sampled. The size (diameter) of the circles is proportional to their coefficient of variation.

The total yields of HC per the weight of 8 mature leaves is a measure of the grams of HC per plant (likely larger than if the entire plant were extracted). In Table 1, the yields range from 0.03 g/ 8 mature leaves (Bozeman, MT) to 1.428 g (Gruver, TX). The highest yields were in the Texas Panhandle (1.20 g - 1.43 g) and Ellsworth, KS (1.0 g) and Enid OK (0.88 g) (Fig. 3). The lowest yields were in the southwestern United States. Note the difference between the San Diego, large leaves (SL, .43 g) and small leaves (SS, .27 g). These are plants collected from the same population. Recall that the % yields were quite similar (Table 1, SS, 4.59%; SL, 4.68%).

The northern-most populations in Montana, North Dakota and Minnesota were generally low in HC yields and that may reflect introgression from cultivated sunflowers (Fig. 3).

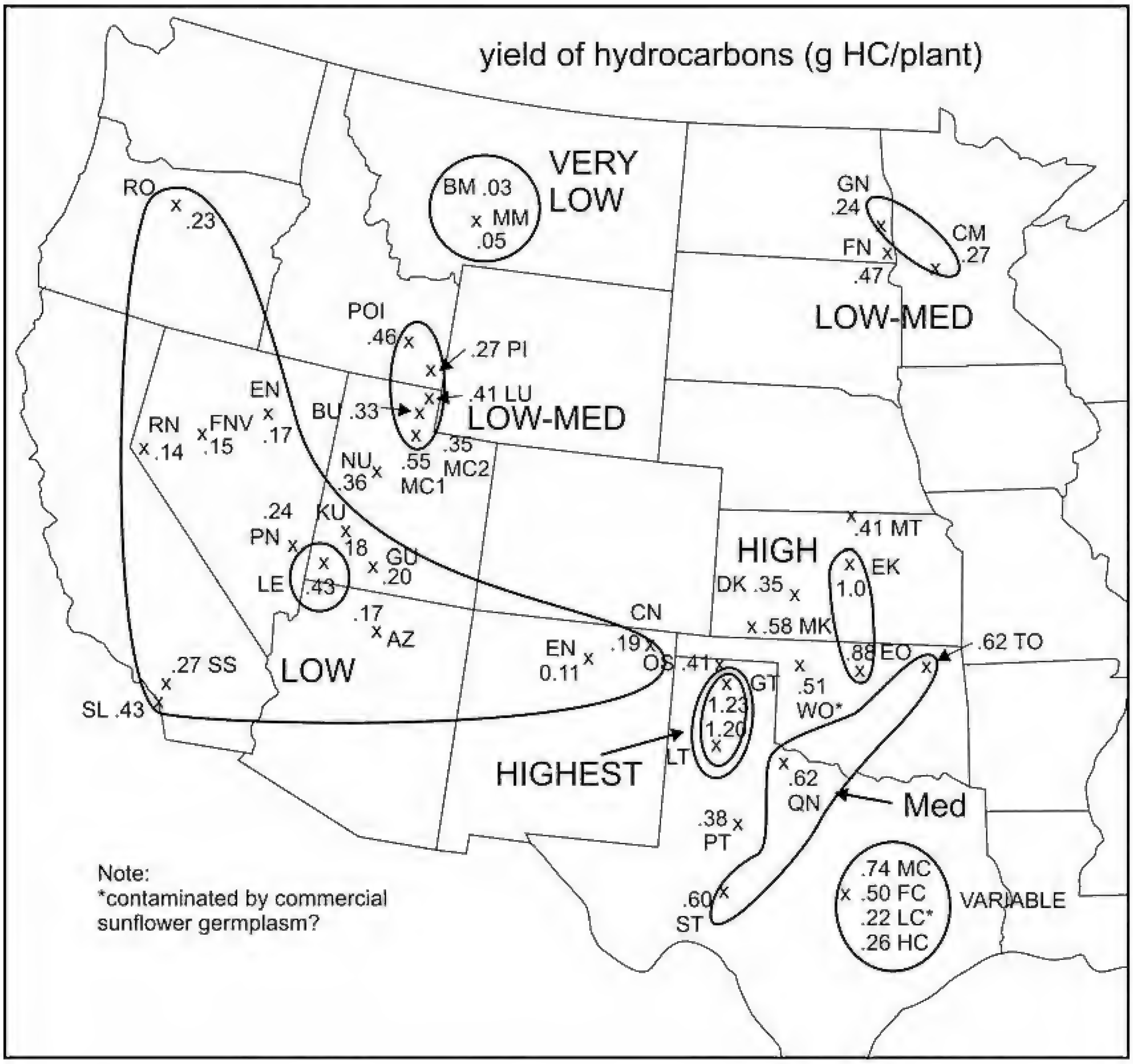

Figure 3. Geographic variation in the HC yields (g/ weight of 8 mature, dried leaves, basis).

This study revealed that the range of variation in HC yields of native sunflowers is quite large, from 1.0 to 12.63%. It is remarkable to find such a wide range, but indicates the potential of *H. annuus* to produce copious amounts of hydrocarbons for use as fuel and in the petro-chemical industry. Many of the highest yielding plants were severely eaten by grasshoppers and covered with black (sugar) ants, feeding on resin extruded from the stem, petioles and leaf bracts. It appears that the high yields were responses to insect damage that induced defense chemicals. Adams et al. (2017b) found that plants grown in a greenhouse from seeds from the high yielding populations (GT, LT) had low to very low yields of HC in lush, protected greenhouse conditions. This seems to confirm the theory of bio-induction of defense chemicals. Additional research is needed to quantify and understand the induction of higher HC yields in *H. annuus*.

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Table 1. Yields of hydrocarbons (HC) *H. annuus*, from natural populations. Coefficient of variation computed as standard deviation / mean.

popn id, sample ids	population sampled	weight 8 lvs	% yield corr'd*	Coef. of variation	Range of yields	yield g/8 lvs
AZ	15021 Camp Verde, AZ	4.48	3.79	33.2%	(1.72-5.56)	0.170
BM	15274 Bozeman, MT	3.28	3.80	25.3	(2.82-5.05)	0.030
BU	15022 Brigham City, UT	5.70	5.90	31.2	(2.90-8.31)	0.336
CM	15287 Clay Co. MN	10.47	2.61	11.2	(1.92-2.95)	0.273
CN	14981 Capulin, NM	3.51	5.29	28.0	(3.16-8.34)	0.186
DK	14940 Dodge City, KS	9.75	3.52	26.2	(2.68,5.49)	0.346
EK	14941 Ellsworth, KS	18.74	5.38	24.0	(3.63,7.35)	1.008
EN	14980 Eagle Nest, NM	4.34	2.62	32.6	(1.17-3.85)	0.114
ENV	15266, Eureka, NV	4.84	3.54	25.3	(2.74-5.56)	0.171
EO	14943 Enid, OK	17.60	4.97	23.9	(3.23,7.55)	0.875
EU	15332 Enterprise, UT bulk,	6.72	5.28	na	na	0.355
FC	14977 Falls Co., Satin, TX	9.49	5.29	14.2	(4.19-6.59)	0.502
FN	15289 Fargo,ND	9.56	4.87	9.3	(4.33-5.77)	0.466
FNV	15265 Fallon, NV	2.17	6.86	28.1	(5.21-11.74)	0.147
GN	15288 Grandin, ND	7.36	3.23	9.1	(2.74-3.71)	0.238
GT1	14952-1 Gruver, TX	16.93	7.26	24.4	(5.01-11.06)	1.231
GT2	14952-2 Gruver, TX	18.03	7.92	19.8	(6.25-10.78)	1.229
GT3	14952-3 Gruver, TX	12.50	8.16	16.4	(7.0-10.51)	1.428
GT4	14952-4 Gruver, TX	14.50	8.60	23.5	(6.52-12.63)	1.020
GU	15262 Glendale, UT	2.95	5.30	44.5	(2.27-10.03)	0.202
HC	14979 Hill Co., TX (Waco area)	5.21	4.92	37.2	(2.61-8.65)	0.263
KU	15270 Kolob Canyon, UT	4.67	3.77	17.0	(2.59-4.53)	0.176
LC	14978 Limestone Co., TX (Waco area)	6.13	3.58	31.3	(2.61-6.25)	0.219
LE	15268, Leeds, UT	6.10	7.01	20.4	(5.7-9.68)	0.428
LT1	14947-1 Lake Tanglewood, TX	16.65	8.47	22.2	(6.73-12.15)	1.410
LT2	14947-1 Lake Tanglewood, TX	13.92	7.31	16.3	(5.63-9.06)	1.018
LU	15023, Logan, UT,	7.57	5.44	25.7	(3.98-8.67)	0.412
MC	14976 McLennan Co., TX, Waco	11.95	6.18	14.4	(4.74-7.76)	0.739
MCU1	15026 Mill Creek, UT 2016	9.54	5.74	26.6	(3.91-8.22)	0.548
MCU2	15273 Mill Creek, UT 2017	6.66	5.25	25.1	(3.43-7.21)	0.350
MK	14939 Meade, KS	10.38	5.64	18.6	(3.91,7.21)	0.585
MT	14982 grown from seed ex Montrose, KS, ex PI 413033	8.44	4.91	8.9	(4.65-5.42)	0.414
MM	15275 MSU, Bozeman, MT	4.47	4.84	29.8	(3.68-5.68)	0.047
NU	15271 Nephi, UT	7.38	4.85	27.8	(1.63-4.03)	0.358
OS	14946 Oslo, TX	6.48	5.95	20.3	(4.19-8.17)	0.406
PI	15024, Preston, ID	4.29	6.30	27.8	(3.91-9.34)	0.270
POI	15025, Pocatello, ID	7.99	5.71	16.0	(4.46-7.55)	0.456
PNV	15267 Pioche, NV	4.58	5.20	33.8	(2.74-8.04)	0.238
PT	14935 Post, TX	8.83	4.36	31.7	(2.33,6.63)	0.385
QN	14936 Quanah, TX	14.41	4.32	23.8	(2.88,5.62)	0.623
RN	15029 Reno, NV	2.87	5.11	30.0	(183-3.82)	0.156
RO	15027 Redmond, OR	5.74	4.06	22.6	(3.37-6.28)	0.233
SC	14953 cult. sunflowers, Oslo, TX	12.41	3.20	13.4	(2.75-3.85)	0.397
SL	14951 San Diego, CA, big leaves	9.04	4.68	21.8	(2.75-6.11)	0.432
SS	14950 San Diego, CA, small lvs.	5.83	4.59	29.2	(2.75-7.51)	0.268
ST	14945 grown from seed, ex Sonora, TX, PI413168	11.47	5.19	32.9	(1.99-7.55)	0.595
TN	15264 Tonopah, NV, 1 plant	7.06	6.39	na	na	0.451
TO	14942 Tulsa, OK	13.64	4.56	23.6	(3.16,6.04)	0.622
WO	14944 Woodward, OK, very large, smooth leaves, cult?	19.64	2.62	15.5	(1.92-3.09)	0.515

*correction factor = soxhlet, 6hr extraction/ hexane 18 hr shaker yield = 2.06

Appendix I Population locations. (see Adams et al. 2017, for populations sampled in 2016).
H. annuus:

GN Common in spots e side of US 89, 1mi. s. of Glendale, flowering, few with seed. Sampled 10 plants for HC. 37° 18' 11" N, 112° 36' 09" W., 5700 ft., 24 Aug 2017, Kane Co., Utah, Coll. *Robert P. Adams 15262*

TN Only one plant found, along highway pavement., US 95, in Tonopah, flowering, 38° 04' 40" N, 117° 15' 04" W., 5830 ft, 28 Aug 2017, Nye Co., Nevada, Coll. *Robert P. Adams 15264*

FNV ~100 plants, along canal ditch, w side of US 95, 8.5 mi s of Fallon, flowering, some seeded, 39° 21' 03" N, 118° 46' 59" W., 3820 ft. Lots of black ants on plants. 28 Aug 2017, Churchill Co., Nevada, Coll. *Robert P. Adams 15265*

ENV ~15 plants, in dry ditch, in pinyon-juniper woodland, N side of US 50, 3.5 mi s of Eureka, flowering, some seeded, 39° 26' 07" N, 115° 55' 22" W., 7040 ft. some black ants on plants. 29 Aug 2017, Eureka Co., Nevada, Coll. *Robert P. Adams 15266*

PNV ~30 plants, in dry ditch, in juniper woodland, W side of US 93, 31.3 mi n of Pioche, flowering, some seeded, 38° 19' 45" N, 114° 36' 38" W., 6130 ft. some black ants on plants, 29 Aug 2017, Lincoln Co., Nevada, Coll. *Robert P. Adams 15267*

LE ~30 plants, in dry ditch and on disturbed Hwy Dept. gravel storage, W side of old US 91, 5 mi S of Leeds, flowering, few seeded, 37° 12' 28" N, 114° 36' 38" W., 3055 ft. some black ants on plants, 9 Sept 2017, Washington Co., Utah, Coll. *Robert P. Adams 15268*

KU ~30 plants, along the pavement in the parking lot of Kolob Canyons National monument, flowering, few seeded, 37° 27' 13" N, 113° 13' 34" W., 5070 ft. , 15 Sept 2017, Washington Co., Utah, Coll. *Robert P. Adams 15270*

NU ~20 plants, flowering, few seeded, along the pavement in the parking lot of Owens-Corning Plant, Nephi, 39° 40' 04" N, 111° 51' 07" W., 5922 ft., 15 Sept 2017, Juab Co., Utah, Coll. *Robert P. Adams 15271*

MCU2 ~20 plants, next to sidewalk, flowering and seedlings, multiple branches. Common along sidewalks, Mill Creek, s side of I80 on 2000 E, east side of 2000E., flowering, few seeded, 40° 42' 56.19" N, 111° 50' 01.98" W., 4539 ft., 15 Sept 2017, Salt Lake Co., Utah, Coll. *Robert P. Adams 15273,* (re-collection, see 2016, Adams 15026, Adams et al. 2017)

BM Gravely roadside, adjacent to wet area with cattail, and Maximilian sunflower, near jct. I90 and frontage RD, E of Bozeman. 0.2-0.5 m tall, BZMT1-5, 45° 40' 30.2" N, 111° 00' 29.4" W., 4810 ft., 15 Sept 2017, Gallatin Co., Montana, Coll Matt Lavin sn, Lab Acc. *Robert P. Adams 15274*

MM Weeds in middle of MSU campus, sporadic with western wheatgrass and quackgrass on dry rocky soil, 0.3-0.5 m tall. MSU1-5, 45° 40' 04.5" N, 111° 03' 01.3" W., 4910 ft., 15 Sept 2017, Gallatin, Co., Montana, Coll Matt Lavin sn, Lab Acc. *Robert P. Adams 15275*

CM West of Felton, MN. Roadside ditch, edge of soybean field. Typical population, approximately 40 plants, 47° 05' 18.2" N, 96° 36' 56.7" W., 895 ft., 23 Aug 2017, Clay Co., Minnesota, Coll. Gerald Seiler, sn, Lab Acc. *Robert P. Adams 15287*

GN East of Grandin, ND, Along edge and in soybean field. Typical population, approximately 50 plants, 47° 12' 15.5" N, 96° 50' 47.11" W., 875 ft., 23 Aug 2017, Cass Co., North Dakota, Coll. Gerald Seiler, sn, Lab Acc. *Robert P. Adams 15288*

FN Typical plants near large drainage ditch, scattered along edge for several hundred feet. Large population that has been there for over 40 years. West side of Fargo, 46° 56' 07.7" N, 96° 51' 34.45" W., 896 ft., 23 Aug 2017, Cass Co., North Dakota, Coll. Gerald Seiler, sn, Lab Acc. *Robert P. Adams 15289*

EU Bulk coll. Enterprise, UT, Washington Co., Utah, along UT hwy 219, in Enterprise, ca. 39°34’24” N, 113°43’59 W, 5330 ft., Coll: *Robert P. Adams 15332*

Screening hydrocarbon yields of sunflowers: *Helianthus maximiliani* and *H. nuttallii* (Asteraceae)

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ABSTRACT

Populations of perennial *Helianthus maximiliani* Schrad. from eastern Oklahoma, central Texas and Montana were examined for their content of free, extractable hydrocarbons (HC) from leaves. The highest yielding population was at Langston, OK (5.06%), and the lowest yield was in Falls Co, TX (2.2%). The coefficient of variation for % HC yield was 24.3 to 30.0% in central Texas, 26.8% (Langston, OK) and very low, 9.5%, in Bozeman, MT. HC yields (g HC/ g DW plant) were variable in central Texas (0.071 - 0.144 g/ plant), lowest at Bozeman, MT (0.042 g/ plant) and high at Langston, OK (0.098 g/ plant). The percent yields of HC from *H. nuttallii* Torr. & Gray were very high (comparable with the annual, *H. annuus*) in the Glendale, UT population (7.02%), high at Sedona, AZ (5.43%) and low near Kanab, UT (3.12%). The coefficient of variation for % HC yield was highest at Kanab, UT (37.7%), moderate at Glendale, UT (16.4%) and lowest at Sedona, AZ (9.5%). HC yields (g HC/ g DW plant) *H. nuttallii* varied from 0.044 g (Kanab, UT), to 0.088g (Sedona, AZ), to 0.125 g (Glendale, UT). The Glendale, UT population of *H. nuttallii* deserves additional examination due to its high yields.
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KEY WORDS: *Helianthus maximiliani, H. nuttallii,* Sunflower, yields of hexane soluble leaf hydrocarbon.

Adams and Seiler (1984) surveyed 39 taxa of sunflowers that were grown in a common garden at the USDA lab, Bushland, TX. They analyzed cyclohexane (hydrocarbon), rubber, methanol (resins) yields plus protein concentrations. They reported (Table 1) cyclohexane (HC) yields of 3.10% (IN), and 3.50% (NM) for *H. maximiliani* and 5.25% (NV) and 5.17% (UT) for *H. nuttallii* (Table 1).

Adams et al. (1986) screened 614 taxa from the western US for their hydrocarbon (hexane soluble) and resin (methanol soluble) yields. They reported 4.15% HC from *H. nuttallii,* but they did not examine *H. maximiliani.*

Table 1. Analyses of 39 Taxa of *Helianthus*, representing 49 accessions. Modified from Adams and Seiler, 1984).

Helianthus species	Annual (A) or perennial (P)	Origin	C-hex fract.	Rubber yield[a]	MeOH fract.	Total	Percent Protein[b]
agrestis	A	Brandenton, FL	7.38	1.66	13.45	20.83	6.9
angustifolius	P	Alvin, TX	3.33	0.18	9.58	12.91	15.9
annuus	A	Winton, OK	7.09	1.40[c]	11.73	18.82	8.7
annuus, hybrid 894	A	Bushland, TX	2.23	0.49	14.65	16.88	8.6
anomalus	A	Mexican Water, AZ	5.74	0.18	12.30	18.04	9.8
agrophyllus	A	Rockport, TX	6.52	1.14[c]	9.60	16.12	11.9
arizonensis	P	Snowflake, AZ	6.13	0.28[c]	13.16	19.29	18.4
califomicus	P	Napa, CA	3.05	1.78[c]	12.44	15.49	13.8
ciliaris	P	Bushland, TX	5.26	0.57	17.17	22.43	15.6
debilis	A	Titusville, FL	1.95	0.68	8.83	10.78	9.6
deserticola	A	Leeds, UT	3.16	0.82	10.96	14.12	5.3
divaricatus	P	Wister, OK	1.09	0.47[d]	11.54	13.44	2.6
glaucophyllus	P	Blowing Rock, NC	3.29	0.25	9.50	12.79	8.1
grosseserratus	P	Cherokee Co., KS	2.36	0.28	12.28	14.64	14.6
grosseserratus	P	Hooker Co., KS	4.41	0.28	14.37	18.78	20.1
grosseserratus	P	Stuart, OK	3.56	0.28	10.49	14.05	17.1
hirsutus	P	Wilburton. OK	1.60	0.30	8.30	9.90	6.1
laciniatus	P	Mimbres River, NM	3.15	0.31	12.40	15.55	9.9
laetiflorus	P	Lyon Co., KS	2.22	0.66	10.64	12.86	11.9
laevigatus	P	Botetourt Co., VA	3.53	na	18.24	21.77	13.9
maximiliani	**P**	**Bloomington, IN**	**3.10**	**na**	**13.21**	**16.31**	**10.8**
maximiliani	**P**	**San Jon, NM**	**3.50**	**0.24**	**9.87**	**13.37**	**15.3**
maximiliani	P	Gatesville, TX	2.53	na	10.30	12.83	8.9
microcephalus	P	Cherokee Co., SC	4.77	0.26[c]	14.25	19.02	14.1
mollis	P	Greenwood Co., KS	3.26	0.31	11.05	14.31	8.9
mollis	P	Okmulgee Co., OK	2.60	0.31	9.72	12.32	8.5
mollis	P	Rivercrest, TX	1.87	0.31	8.58	10.45	6.6
neglectus	A	Kennit, TX	3.83	0.10	11.71	15.54	16.2
nuttallii	**P**	**Orovada, NV**	**5.25**	**0·96[c]**	**10.23**	**15.48**	**8.8**
nuttallii	**P**	**Payson, UT**	**5.17**	**na**	**12.76**	**17.93**	**10.6**
occidentalis	P	Raymondville, MO	2.12	0.48	15.14	17.26	11.9
occid. ssp. plantagineus	P	Sheridan, TX	2.36	1.62	18.33	20.69	8.8
paradoxus	A	Ft. Stockton, TX	3.46	0.15	19.54	23.00	13.3
petiolaris ssp.fallax	A	Adrian, TX	2.15	0.30	11.99	14.14	17.3
petiolaris ssp. petiolaris	A	Memphis, TX	1.86	0.14	21.00	22.86	12.1
praecox ssp. hirtus	A	Carrizo Springs, TX	5.19	0.49	10.05	15.24	13.8
pumilis	P	Boulder, CO	1.72	0.53	6.87	8.59	7.4
resinosus	P	Collins, MS	2.89	1.78[c]	11.76	14.65	11.9
rigidus ssp. rigidus	P	Brookston, IN	1·86	na	9.93	11.79	7.8
rigidus ssp. subrhomboides	P	Leyden, CO	1·42	na	10.90	12.32	9.9
salicifolius	P	Kansas	3.13	0.37	9.30	12.43	7.1
salicifolius	P	Muenster, TX	3.26	0.37	9.31	12.57	11.2
silphioides	P	Wister, OK	2.63	0.42	18.01	20.64	10.0
simulans	P	Milton, FL	3.42	0.31	13.91	17.33	18.1
smithii	P	Morgantown, NC	4.48	0.58[c]	11.77	16.25	12.2
strumosus	P	Siler City, NC	2.98	0.55	11.80	14.78	12.9
tuberosus	P	Kilgore, TX	2.26	0.93	13.28	15.54	12.1
tuberosus x annuus	P	Turlock, CA	1.73	na	12.21	13.94	9.3
Average			3.39	0.57	12.26	15.65	11.35

a Rubber yields, for leaves except for *divaricatus,* are from Stipanovic *et al* 11 22
b Protein determined by Kjeldahl N x 6-25.
c By 13C·NMR spectral analysis. All others by gravimetric.
d Whole plant analyzed.

Seiler, Carr and Bagby (1991) reported on 28 *Helianthus* taxa for their yields of oil, polyphenols, protein and rubber. The rubber was found to be of lower molecular weight than *Hevea* rubber, but still appeared to be useful as a plasticizing additive and for coatings inside pipes and containers.

Yields of natural rubber has recently been reported for *H. annuus* (Pearson et al. (2010a) that ranged from 0.9% to 1.7% rubber in cultivated sunflowers (Fig. 4, Pearson et al. 2010b).

Most recently, Adams et al. (2018) reported on geographical variation in natural rubber yields in *H. annuus*. They found considerable variation in yields of natural rubber with the highest yielding populations at Mill Creek, UT and in the Waco, TX area. These high yielding populations are in very different eco-systems with different climates and soils. The rubber concentrations in adjacent plants sometimes varied from none to 16 mg/g.

Recently, Adams et al. (2017a) reported on hydrocarbon yields (HC) from a large area from Oklahoma to southern California. This report is a continuation of that survey of HC in sunflowers as we examine HC yields on *Helianthus maximiliani* Schrad. and *H. nuttallii* Torr. & Gray (Adams and TeBeest, 2016; Adams, et al. 2016; Adams et al. 2017b).

MATERIALS AND METHODS

Population locations - see Appendix I.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays. The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared. Yields were corrected by a correction factor derived from soxhlet, 6 hr extraction with hexane yields (3) divided by 18 hr shaker with hexane yield(3) = 2.06.

RESULTS

The % HC yields ranged from 2.2 to 5.06% (Table 2) for *H. maximiliani*. The highest yielding population was at Langston, OK (5.06%), and the lowest yield was in Falls Col, TX (2.2%). Yields from populations in central Texas, around Waco, TX, were moderate in % HC yields from 2.20 to 3.68% (Table 2). The Waco, TX yields were comparable to that reported (Adams and Seiler, 1984) (see Table 2, *H. maximiliani*, 3.10%, 3.5%). The population at Langston, OK (5.06%) was higher than previously reported for *H. maximiliani* (Table 1).

The coefficient of variation for % HC yield was 24.3 to 30.0% in central Texas, 26.8% (Langston, OK) and a very low 9.5% in Bozeman, MT (Table 2). The low variation at Bozeman may be due to a small population size.

HC yields (g HC/ g DW plant) were variable (Table 2) in central Texas (0.071 - 0.144 g/ plant), lowest at Bozeman, MT (0.042 g/ plant) and high at Langston, OK (0.098 g/ plant). The low HC g yield was largely due to the very small leaves (1.14 g for 8 leaves). Bozeman is near the northern limit of *H. maximiliani*, and, as such, may just produce very small leaves due to the short growing season.

The percent yields of HC from *H. nuttallii* (Table 2) were very high (comparable with the annual, *H. annuus*) in the Glendale, UT population (7.02%), high at Sedona, AZ (5.43%) and low near Kanab, UT (3.12%). The yield from Sedona, AZ (5.43%) is comparable to those reported (Adams and Seiler, 1984) for plants from NV and UT (5.25, 5.17%, respectively, Table 1).

The *H. nuttallii* at the Kanab site has few plants and they grow in the water of a small pond that has afternoon shade and is spring fed in an isolated canyon. This was a very mesic, and lush site that may have resulted in lower amount of stored defense chemicals than in plants less protected (as is the case of the Glendale population).

The coefficient of variation for % HC yield was highest at Kanab, UT (37.7%), moderate at Glendale, UT (16.4%) and lowest at Sedona, AZ (9.5%) (Table 2). The large amount of variation at the Kanab site is surprising (see habitat discussion above). The low amount of variation at the Sedona site may be due to population size.

HC yields (g HC/ g DW plant) *H. nuttallii* varied from 0.044 g, Kanab, UT, to 0.088 g, Sedona, AZ, to 0.125 g, Glendale, UT (Table 2) .

The Glendale, UT population of *H. nuttallii* is in a mesic / seasonally dry roadside ditch with grasses and cattails. It extends for about a kilometer and has thousands of plants. This population deserves additional research with seed-grown progeny and transplant studies due to its high HC yields (in progress).

Table 2. The yields of hydrocarbons (HC) for *H. maximiliani* and *H. nuttallii*.

popn id, sample ids	collection number, taxon, location	wt / 8 lvs	% HC yield	Coef. of var. %	Range of yields	HC g/ plant (8 lvs)
	H. maximiliani					
MxCC	15340, *H. maximiliani,* Coryell Co., Holmes,	3.40	3.24	30.0	2.17-5.42	0.110
MxFC	15341, *H. maximiliani,* Falls Co., Holmes,	3.23	2.20	24.3	1.19-3.17	0.071
MxMC	15342, *H. maximiliani,* McLennan Co., Holmes	3.90	3.68	24.7	2.54-5.42	**0.144**
MXMT	15276, *H. maximiliani,* Bozeman Lavin	1.14	3.71	9.5	3.32-4.47	0.042
MXOK	15333, *H. maximiliani,* Langston, OK, Hart	1.94	**5.06**	26.8	3.16-7.82	0.098
	H. nuttallii					
NuKUT	15263, *H. nuttallii,* Kanab, UT Adams	1.40	3.12	37.7	1.7-5.26	0.044
NuGUT	15260, *H. nuttallii,* Glendale UT Adams	1.78	**7.02**	16.4	5.58-9.16	**0.125**
NuSAZ	15290, *H. nuttallii,* Licher, Sedona, AZ,	1.62	5.43	9.5	4.49-6.07	0.088

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Appendix I Population locations.

Helianthus maximiliani Schrad.

MxMT Wet area with cattail and Bebbs willow, near jct. I90 and frontage RD, E of Bozeman. 1-2m tall, common at this site, in wets areas they dry out by middle summer. 45° 40' 29.8" N, 111° 00' 29.4" W., 4810 ft., 15 Sept 2017, Gallatin Co., Montana, Coll Matt Lavin sn, Lab Acc. *Robert P. Adams 15276*

MxMc 1.0 mile southwest of Old Robinson Road, Robinson, 31° 28' 10.69" N, 97° 09' 00.3423" W., 7 Oct 2017, McLennan Co., Texas, Coll: Walter Holmes sn, Lab acc.: *Robert P. Adams 15305*

MxFC Jct of US Hwy 77 and TX Hwy 7, 1.35 miles northwest of Chilton. 31° 17' 26.30" N, 97° 03' 33.91" W., 9 Oct 2017, Falls Co., Texas, Coll: Walter Holmes sn, Lab acc.: *Robert P. Adams 15306*

MxCC 12.1 miles east of Gatesville on US Hwy 84, at jct of Tex. Hwy 185, 31° 26' 01.04" N, 97° 32' 24.26" W., 13 Oct 2017, Coryell Co., Texas, Coll: Walter Holmes sn, Lab acc.: *Robert P. Adams 15309*

MxOK Along dirt road. 35° 53' 10.73" N, *97° 13' 47.21" W. Oct 2017, Logan Co., Oklahoma, Coll: Steve Hart sn, Lab acc.:* Robert P. Adams *15310*

Helianthus nuttallii Torr. & Gray

NuGUT Ca. several hundred plants, most flowering now. 3-4 ft tall. in a swale (wet area from excess irrigation water) on the west side of US 89, 2 mi n Glendale, with wild cane, milkweed, in seasonally wet grass ditch. 10 plants sampled for HC. 37° 20' 44" N, 112° 36' 04" W., 5960 ft., 24 Aug 2017, Kane Co., Utah, Coll. *Robert P. Adams 15260*

NuKUT Ca. 50 plants, most flowering now. 4-5 ft tall. on sandy on edge of perennial pond at Best Friends Center. 10 plants sampled for HC. 37° 08' 22" N, 112° 32' 32.5" W., 5350 ft, 24 Aug 2017, near Kanab, Kane Co., Utah, Coll. *Robert P. Adams 15263*

NuSAZ Near Indian Gardens, Oak Creek Canyon, n of Sedona. edge of marshy area in outflow form spring, 34° 54' 22.11" N, 111° 43' 35.24" W., 4580 ft., 4 Sept 2017, Coconino Co., Arizona, Coll. Max Licher, sn, Lab Acc. *Robert P. Adams 15290*

Comparison of mortality of *Quercus stellata* (post oak) after a Central Texas drought

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ABSTRACT

A one-year drought in central Texas changed the structure of a *Quercus stellata* community. The density of live *Q. stellata* trees decreased by 22% and the density of standing dead increased by 7.9 times from eight dead to 63 trees/ha dead in 2012 after the drought. The total live plus standing dead trees did not change. Before the drought, dead trees were in the 22-31 cm diameter size class and after the drought in 2012, they were mostly between the 15 and 29 cm diameter size classes. No live or dead trees were found < 9 cm in diameter before the drought and no live trees and only two standing dead tree <18 cm in diameter were found after the drought. Understory woody vines, shrubs and juvenile trees decreased by 70% after the drought. *Quercus stellata* juveniles decreased by 84% to a density of 300 plants/ ha after the drought. Woodland or savanna communities can change rapidly after shifts in environmental conditions, but direction of the changes are difficult to predict. Published on-line www.phytologia.org *Phytologia 100(3): 1-5 (Sep 21,, 2018).* ISSN 030319430.

KEY WORDS: community structure, density, basal area, drought effects, standing dead

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Aridity and drought are not the same. Aridity is related to climate and describes long term dry conditions while drought is relatively short term and concerns a temporary state of dryness of the weather (Mishra and Singh 2010; Djebou and Clement 2017). Deserts are dry, but the climate is described as arid while other biomes have more rainfall than deserts but have periods of dryness or droughts. The consequences of aridity and drought on plant communities varies due to the physiological and morphological characteristics of the plants tolerance to dry conditions.

The central Texas Edwards Plateau Region extends about 440 km east to west from the San Antonio-Austin area, with an east to west rainfall gradient with a mean of 84 cm/yr in the east and 38 cm/yr in the west. Rainfall is not equally dispersed and there are usually peaks in May and September with very little in June, July and August (National Climatic Data Center 2012). The annual rainfall can be misleading, because means have varied from 13 to 132 cm/yr (5 to 52 inches/yr) in the east with successive years of below or above average rainfall.

Plant communities, including woodland or savanna communities, can change rapidly especially after stressful or unusual conditions including droughts (Allen and Breshears 1998; Breshears et al. 2005; Kane et al. 2011).The Edwards Plateau Region in the past was more grassland and savanna, but is now known for its high density and basal area of *Juniperus ashei* (mountain cedar, cedar or ashe juniper) and *Quercus fusiformis* (Escarpment or Hill Country live oak) (Van Auken 1988). In addition to *Q. fusiformis*, other fairly numerous oak species found in various places in the region are *Q. buckleyi* (Texas oak), *Q. laceyi* (Lacey oak), *Q. marilandica* (blackjack oak), *Q. shumardii* (Shumard oak), *Q. muehlenbergii* (chinkapin oak) and *Q. stellata* (post oak)(Texas Forest Service 2018).

We have been observing a *Q. stellata* population in the Edwards Plateau for over seven years (Bush and Van Auken 2015), and as with many *Quercus* species in North America, this central Texas community seems to be changing. Changes appear to be widespread across North America and involve many *Quercus* species (Lorimer 1992; Lorimer et al 1994). There is evidence for a lack of recruitment of mature *Quercus* trees (Shumway et al. 2001; Ryniker et al. 2006; Cowell and Hays 2007). This recruitment failure could be caused by erratic seed production, fruit consumption, browsing damage, decreased fire frequency, low-light conditions under the canopy, and/or climatic variability (Abrams 2003). These features do not seem limiting, but there is still reduced recruitment into the adult populations (Lorimer 1992; Lorimer et al 1994). However, drought effects have not been examined.

Few *Quercus stellata* communities have been reported in the eastern part of the central Texas Edwards Plateau region (Correll and Johnston 1979; USDA 2018). Texas drought history had been reconstructed for the past 300 years by aging and comparing *Q. stellata* growth rings (Stahle and Cleaveland 1988), but community structure has not been examined until recently. We previously compared a managed and a non-managed *Q. stellata* community and found major ecological differences (Bush and Van Auken 2015). One community did not have any *Juniperus ashei* trees because of management (removal) and a higher *Q. stellata* density but lower total community density. The non-managed community had a total community density ten times higher than the managed community because of the presence of *J. ashei* trees. Basal area of *Q. stellata* was not significantly different, but total community basal area increased 41% because of the presence of *J. ashei*. Understory juvenile woody species density was also different, with total density 5.3 times higher in the managed community mostly because of the density of seedlings of juvenile *Ulmus crassifolia* plants (fall elm or cedar elm). In addition, *Q. stellata* juvenile density was fifteen times lower in the unmanaged community.

In October of 2010, a 12 month drought began (National Climatic Data Center 2012). The October rainfall for Boerne, Texas (adjacent to the study site) was 14% of the monthly average from 1946-2011. From October 2010 through September 2011 monthly rainfall for Boerne, Texas was well below the average (from 1946-2011). A general survey of Texas estimated between 100 and 500 million trees were killed by the 2010-2011 drought (2 to 10% of probably 4.9 billion trees in Texas, Edgar 2012). Anecdotal observations suggested increased mortality among established *Q. stellata* in the community on the Cibolo Preserve in Boerne, Texas occurred during this time, but was not quantified.

PURPOSE

The purpose of this study was to compare one *Q. stellata* community at two time periods. The time periods were pre- and post-drought. Phytosociological data from the *Q. stellata* community collected prior to the drought that started in 2010 will be compared to phytosociological data collected from the same place in the *Q. stellata* community but collected in May of 2012 after the one-year drought.

SPECIES

Quercus stellata Wangenh. (post oak), the species studied, is a moderate-sized deciduous tree found from central Texas to the Atlantic Ocean in the east, north to Massachusetts and in the central U.S. north to Kansas (Correll and Johnston 1979; USDA 2018). In Texas, it is found in east Texas westward into the eastern part of the central Texas Edwards Plateau. It may form relatively open, upland savanna or woodland, usually on sandy soils. It has been studied in the more eastern and northern parts of its range (Sims 1988), but the few central Texas *Q. stellata* populations are mostly unstudied. It is reported in the Cross-timbers region of north Texas along with other *Quercus* species in scattered populations. Mixed with these *Quercus* populations is *J. ashei* that is present throughout most of central Texas (Van Auken 2016). In the northeastern area called the Lampasas Cut-Plain, it is found in woodland and forested areas with other *Quercus* species (Gehlbach and Amos 1988).

METHODS

Characteristics of the woody vegetation in the 20 ha woodland portion of a managed 30 ha *Q. stellata* savanna were examined. The community was on the 250 ha Cibolo Preserve in Kendall County, central Texas (29.7691ºN and 98.6935ºW).The initial phytosociological study (Bush and Van Auken 2015) was carried out in May of 2010 using the quadrat procedure (Van Auken et al. 2005). Twenty-five square meter quadrats (5 m x 5 m) were established along a belt transect line within the *Q. stellata* woodland. Two-hundred four quadrats were used to sample trees in 2010 and 120 quadrats were used in 2012. The diameter of each adult tree, live or dead (3 cm in circumference at 1.57 m), was measured with a tree caliper and recorded in each 25 m^2 quadrat. Density and basal area were calculated in order to describe the plant community. In the understory, juveniles (< 0.5 m in height or >0.5 m in height but< 3 cm in circumference) were counted in 1020, 1 m^2 quadrats in 2010 and in 2012, juvenile plants in 600, 1 m^2 quadrats were counted and density was calculated. For overstory trees, density stabilization curves indicated sample adequacy for both sampling times (see Van Auken et al. 2005). From the tree data, diameter size class histograms were constructed and total number as well as live and dead trees in each size class is presented (Ryniker et al. 2006).

RESULTS

There were only three tree sized species found in the overstory of the community studied and only *Q. stellata* density and basal area were examined and presented in the present study (Table 1). The number of live, dead and total *Q. stellata* stems found were counted and the density and basal area were calculated (Table 1). There were only four dead trees found and measured the first year of the study, but 19 dead trees were found and measured after the drought in 2012 (presented as density/ha). Total density of *Q. stellata* trees (live and standing dead) was inspected before and after the drought. Density was 218 and 227 trees/ha respectively but only a slight increase in density (+4% difference in 2012, Table 1). However, the density of live trees in 2012 decreased 22% and the density of standing dead trees increased 7.9 times to 63 stems/ha (Table 1). There was a 3% decrease in total *Q. stellata* basal area (live and dead) in 2012 (Table 1). The live *Q. stellata* basal area decreased by 25%, but the *Q. stellata* standing dead basal area increased 6.7 times (Table 1).

Prior to the drought, the four dead trees were in three of the mid-sized classes examined (Figure 1). After the drought, size class distributions indicated that the small to average sized *Q. stellata* trees size classes (15-33 cm diameter) had most of the mortalities (Figure 2). The largest tree found in the community after the drought was present prior to the drought (Figures 1). Other live, large *Q. stellata* trees were found before the drought, but not after the drought probably because of a smaller number of quadrats sampled after the drought. All of the smallest trees examined after the drought were dead (Figure 2). There were a few trees that died in the larger size classes, but only 8% of the total.

The understory density of woody species also changed after the drought (Table 2). There were 11 woody species including woody vines, shrubs or juvenile trees found in the understory prior to the drought in 2010 and 10 species of woody plants found in the understory of the community after the drought in 2012 (Table 2). There was an overall 70% decrease in density of the understory woody species from 65,549 plants/ha to 19,050 plants/ha with most species declining after the drought (Table 2). *Ulmus crassifolia* (cedar elm) juveniles were the most common woody species both before and after the drought. Juveniles of *Q. stallata, the* dominant overstory species decreased by 84 % to 300 plants/ha after the drought.

DISCUSSION

It has been suggested that the 2010-2011 drought caused the death of 2 to 10% of the estimated 4.9 billion trees in Texas. This information is hard to confirm especially for *Q. stellata* communities. In the present study, we took advantage of a previous, recent survey (pre-drought) to estimate the actual effects of the drought on a *Q. stellata* community by re-sampling the population immediately after the drought. We report a decrease of 22% of live trees in a *Q. stellata* community in central Texas, or a loss of approximately 1,260 *Q. stellata* trees in the 20 ha woodland part of the 30 ha savanna. We were able to confirm anecdotal observations that suggested increased mortality among established *Q. stellata* in the community on the Cibolo Preserve in Boerne, Texas. Other droughts have occurred here in the past including a major drought in the mid-1950s, but historical data of *Q. stellata* mortality or juvenile recruitment after this drought was not reported (Stahle and Cleaveland 1988).

Drought or short term lack of water is a major limiting factor for plant growth and production. Many *Quercus* species have well documented morphological, biochemical, and physiological adaptations to drought (see Abrams 1990). Morphologically, many species of *Quercu*s have leaf curling (Abrams 1990), and at least one species of *Quercus*, *Q. douglasii*, shows leaf abscission during drought (Griffin 1973).

Physiologically, plants may show decreases in photosynthetic rate and growth, a reduction in stomatal conductance, cell dehydration, and an increase in chlorophyll degradation in response to drought. However, many *Quercus* species have more favorable responses to drought than co-occurring species. Under drought conditions several *Quercus* species have higher photosynthetic rates, smaller decreases in photosynthetic rates with drought, higher water use efficiency, and later stomatal closure than co-occurring species (Hinckley et al. 1979; Reich and Hinckley 1980; Bahari et al. 1985; Abrams 1990; Reich et al. 1990; Manes et al. 2006; Cotrozzi et al. 2017; Jafarnia et al. 2018). Many of these responses are also more favorable during drought for those species of *Quercus* that occur in drier areas when compared to their more mesic counterparts; and similarly, individuals for a given species from drier areas have more favorable responses than individual from the same species in wetter areas (Cotrozzi et al. 2017; Jafarnia et al. 2018). Although drought tolerance of many *Quercus* sps. are well documented (see Abrams 1990), it is also well-known that drought can influence the structure and replacement dynamics of many *Quercus* plant communities globally.

Increased tree mortality due to drought and heat has been shown in a number of studies where a *Quercus* sp. was the dominant or co-dominant (Allen et al. 2010). A dieback in *Q. robur* in Italy resulted from a shift from wetter to warm and dry summers (Colangelo et al. 2018). Similar information for other species of *Quercus* has been reported in Turkey, France, Poland, and Spain (see Allen et al. 2010). In North American, *Quercus* dieback due to drought has been shown for the upland temperate mixed forest in the southeast, northeast, and mid-west (Kessler Jr 1989; Starkey and Oak 1989; Starkey et al. 1989; Stringer et al. 1989; Tainter et al. 1990; Clinton et al. 1993; Jenkins and Pallardy 1995; Lawrence et al. 2002; Oak et al. 2004; Starkey et al. 2004; Heitzman et al. 2007), and in a savanna in Minnesota (Faber-Langendoen and Tester 1993). While our study adds to these documented dieback events for *Quercus* species, it is not known what the impact of this dieback will have on the long-term *Q. stellata* community structure.

Some studies have documented long-term changes in *Quercus* community structure. The drought tolerant *Q. pyrenica* displaced *Pinus sylvestris* as a result of droughts and shifts in climate (Fernández-de-Uña et al. 2018). As with most mortality events, drought is not the only factor to consider when determining long-term effects of tree mortality. We previously examined the replacement dynamics of *Q. stellata* in this same community and found little potential for replacement of mature trees as indicated by the lack of juveniles (Bush and Van Auken 2015).This phenomenon has been described for other *Quercus* species across North American forests since the end of the last glacial (Delcourt et al. 1983; Delcourt and Delcourt 1985; Betancourt et al. 1990; Russell and Fowler 2002; Abrams 2003; Aldrich et al. 2005; Ryniker et al. 2006; Cowell and Hayes 2007; MacDougall 2008). Various reasons have been cited for recruitment failure (see Ryniker et al. 2006) including abiotic or biotic factors such as light levels, and competition or herbivory by various animal populations (Lorimer et al. 1994; Nathan and Muller-Landau 2000; Collins 2003; Haas and Heske 2005; Van Auken 2009; Van Auken and Bush 2009). It has been well documented that as water becomes more limiting, plant competition will increase (see Fowler 1986).

One of the greatest challenges for plant ecologist is to understand how tree mortality will affect the composition of most forests under climate change scenarios (Anderegg et al. 2012). While we have documented the increased mortality of *Q. stellata* as a result of drought in this community, it is not known if drought or other factors such as competition, disease, and herbivory are compensatory or additive.

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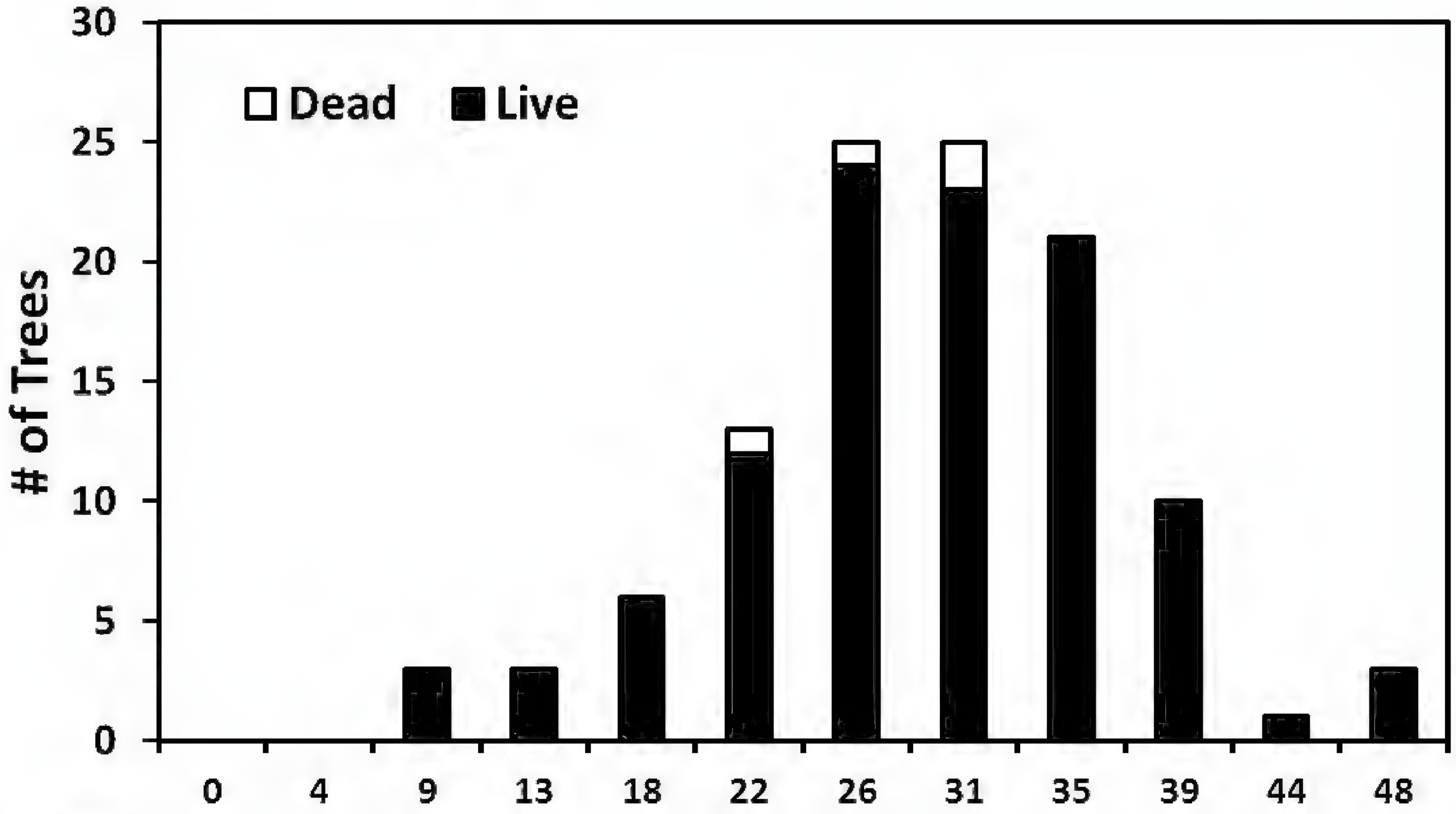
Table 1. Density and basal area before and after a drought for all *Quercus stellata* trees (live, dead, and total) in woodland at the Cibolo Preserve, Kendall County, Texas are presented. Change values are differences between 2010 and 2012 are percent values or times larger or smaller than the 2010 value. Data was collected in May of 2010 and 2012, before and after one-year drought.

	Status	**Density** (trees/ha)	**Basal Area** (m^2/ha)
2010	Live	210	16.7
	Dead	8	0.6
	Total	218	17.2
2012	Live	163	12.6
	Dead	63	4.0
	Total	227	16.7
Change	Live	-47 (-22%)	-4.1 (-25%)
	Dead	55 (7.9 x)	3.4 (6.7 x)
	Total	9 (4%)	0.5 (-3%)

Table 2. Phytosociological data of woody juveniles (< 0.5 m in height) for the *Quercus stellata* woodland part of a savanna located at the Cibolo Preserve, Kendall County, Texas is presented. One thousand and twenty 1 m^2 were examined in 2010 and 600, 1 m^2 quadrats were examined in 2012. Data was collected in May of 2010 and 2012, prior to and following a one-year drought.

	2010	**2012**	
Species	**Density**	**Density**	**% or X Change**
Ulmus crassifolia	21000	11017	-48
Smilax bona-nox	6389	4317	-27
Celits laevigata	2389	1867	-68
Quercus stellata	1833	300	-84
Juniperus ashei	222	50	-77
Diospyros texana	194	267	+38
Ilex deciduas	111	0	-100
Sideroxylon lanuginosum	56	50	-11
Berberis trifoliata	56	0	-100
Parthenocissus quinquefolia	28	183	+6.5x
Quercus laceyi	633	0	-100
Cretaegus sp.	0	267	+100
Prosopis glandulosa	0	100	+100
Total	**64549**	**19050**	**-70**



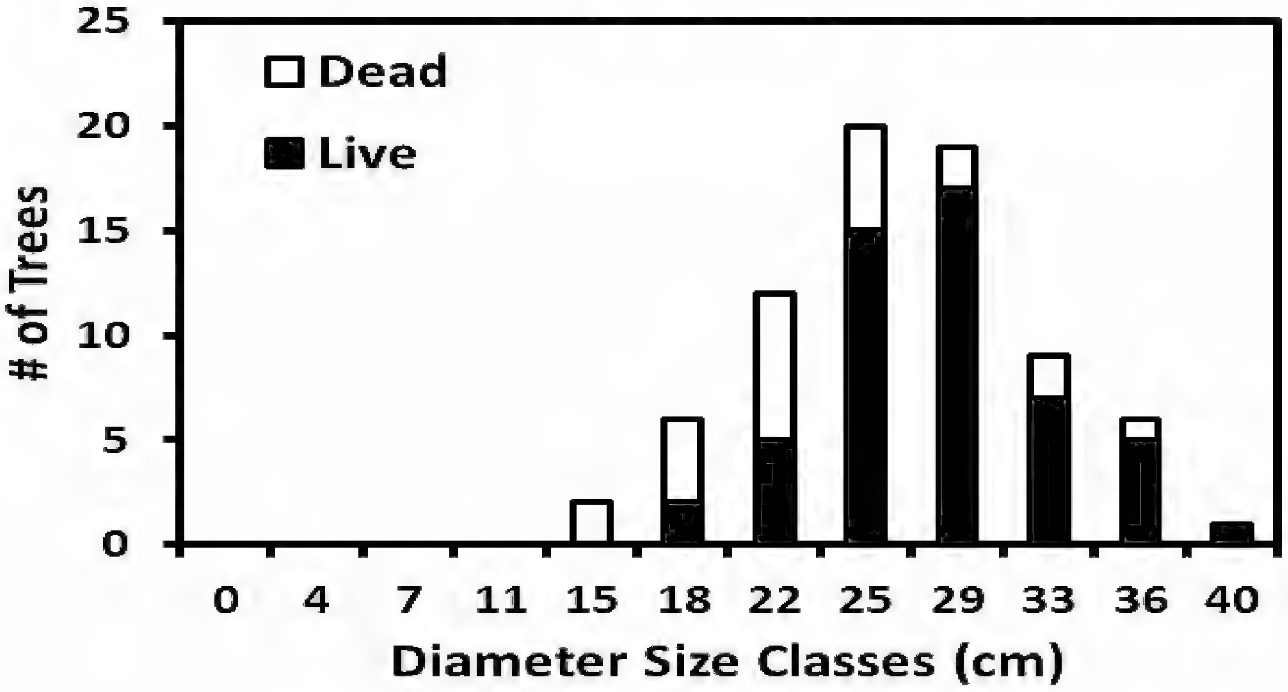


Figure 1 (upper). Diameter size class histogram for a *Quercus stellata* community at Cibolo Preserve, Kendall County, Texas for data collected in May 2010 before a one-year drought.

Figure 2 (lower). Diameter size class histogram for a *Quercus stellata* community at Cibolo Preserve, Kendall County, Texas for data collected in May 2012 after a one-year drought. Notice the lack of small diameter and very large diameter trees in the May 2012 graph(lower) vs. the 2010 (before drought, upper).

The effects of methyl jasmonate on the growth and yields of hydrocarbons in *Helianthus annuus* (Asteraceae, Sunflowers)

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ABSTRACT

Sunflowers, *H. annuus* cv. Little Becka and cv. Munchkin, were sprayed with 100 µM methyl jasmonate (MeJA) to examine the effects MeJA on the free, stored hydrocarbons concentrations. For Little Becka, the % HC yields declined from the control in 2d, further in 4d. Biomass, as estimated by dry weight of 10 leaves/ plant, increased in day 2 and again in day 4 samples. HC yields, as g HC/ g DW 10 leaves, did not differ between control and day 2, but then had a significant drop in day 4. Punching 3 holes per leaf did not effect % HC or g HC, but did result in a significant decline in leaf biomass. For Munchkin, the application of MeJA had no effects on leaf biomass for up to 7 days, then a significant increase after 14 days. It seems likely that this delayed growth was due to normal growth, after the MeJA had dissipated. The % HC yields declined after just 2 days and remained low through day 7, then increased to, statistically, the same level as the control. A similar pattern was seen in g HC/ g DW 10 leaves. MeJA had significant effects on both Little Becka and Munchkin, decreasing the free HC by over 50% in 2 to 4 days, seemingly implying that the application of this growth regulator is not useful in increasing the free HC for alternative fuels from sunflowers. Published on-line www.phytologia.org *Phytologia 100(3):177-182 (Sep 21, 2018).* ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, methyl jasmonate, effects on hydrocarbon yields.

In a seminal paper on the induction of sesquiterpene lactone (STL) defenses in *Helianthus annuus*, by surface application of methyl jasmonate (MeJA), Rowe, Ro and Rieseberg (2012) found that MeJA treated sunflower plants had a lower STL production and lower glandular trichome density. This is in contrast to other studies that have found MeJA to induce increased concentrations of terpenoids in cotton (*Gossypium hirsutum*, Opitz, Kunert and Gershenzon, 2008), *Tanacetum parthenium* (Majdi et al. 2015) and see review on the roles of MeJA in plants by Browse (2005).

It appears that defense chemicals are both constitutive and inducible defenses (see Wittstock and Gershenzon, 2002 for discussion). Recently, we reported (Adams et al. 2017c) that progeny of high hydrocarbon (HC) yielding sunflower (*H. annuus*) populations displayed much reduced HC yields when grown in greenhouse conditions. Notice (Fig. 1) that the percent HC (greenhouse / field grown HC yields) decreased to 45.9, 55.6 and 78.3%. In addition, g HC / g DW leaves was very reduced to 6.1 to 17.9% in greenhouse grown plants. It appears that biotic and abiotic factors in natural populations can have large effects on HC yields. With this in mind, it seemed of interest to investigate the effects of MeJA on HC yields from greenhouse grown sunflowers.

This is a part of a continuing study on the development of sunflowers as a source for natural rubber and bio-fuels from the biomass (Adams et al., 1986; Adams and Seiler, 1984; Adams and TeBeest, 2016; Adams et al. 2016; Adams and TeBeest, 2017; Adams et al. 2017a,b,c; Adams et al. 2018a,b,c; Pearson et al., 2010a,b; Seiler, Carr and Bagby, 1991,).

MATERIALS AND METHODS

Seeds of *H. annuus* cv. Little Becka and cv. Munchkin were obtained from Burpee Seed Co., Warminster, PA and Sunflower Selections, Inc., Woodland, CA, respectively. Seeds were planted in 6 " square plastic pots using Miracle Grow® potting soil. Plants were grown in a growth chamber with LED lighting approximately equal to daylight for 16 hr light, 8 hr dark cycles and watered as needed.

The lowest growing, non-yellowed, 10 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays when the HC yields are reported as highest for sunflowers (Adams et al. 2016). The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Rowe, Ro and Rieseberg (2012) used 100 μM methyl jasmonate (Sigma-Aldrich, Ontario, Can), sprayed on leaves until wet. Their procedure was followed in this study. Adams et al. (2016) have shown that the maximum yields of HC occurs at first flower (R5.1), so all sampling was done at first flower for Little Becka and Munchkin plants.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was filtered through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pre-weighed aluminum pan with concentrated hydrocarbon extract was weighed and tared. Extraction of identical samples by shaking and soxhlet (8 hr) yielded a correction factor of 1.9 (soxhlet yield/ shaking yield), which when corrected to oven dry weight basis (ODW) by 1.085 resulted in a total correction factor of 2.06.

ANOVA and SNK (Student Newman-Keuls) multiple range tests were programmed following the formulations in Steel and Torrie (1960).

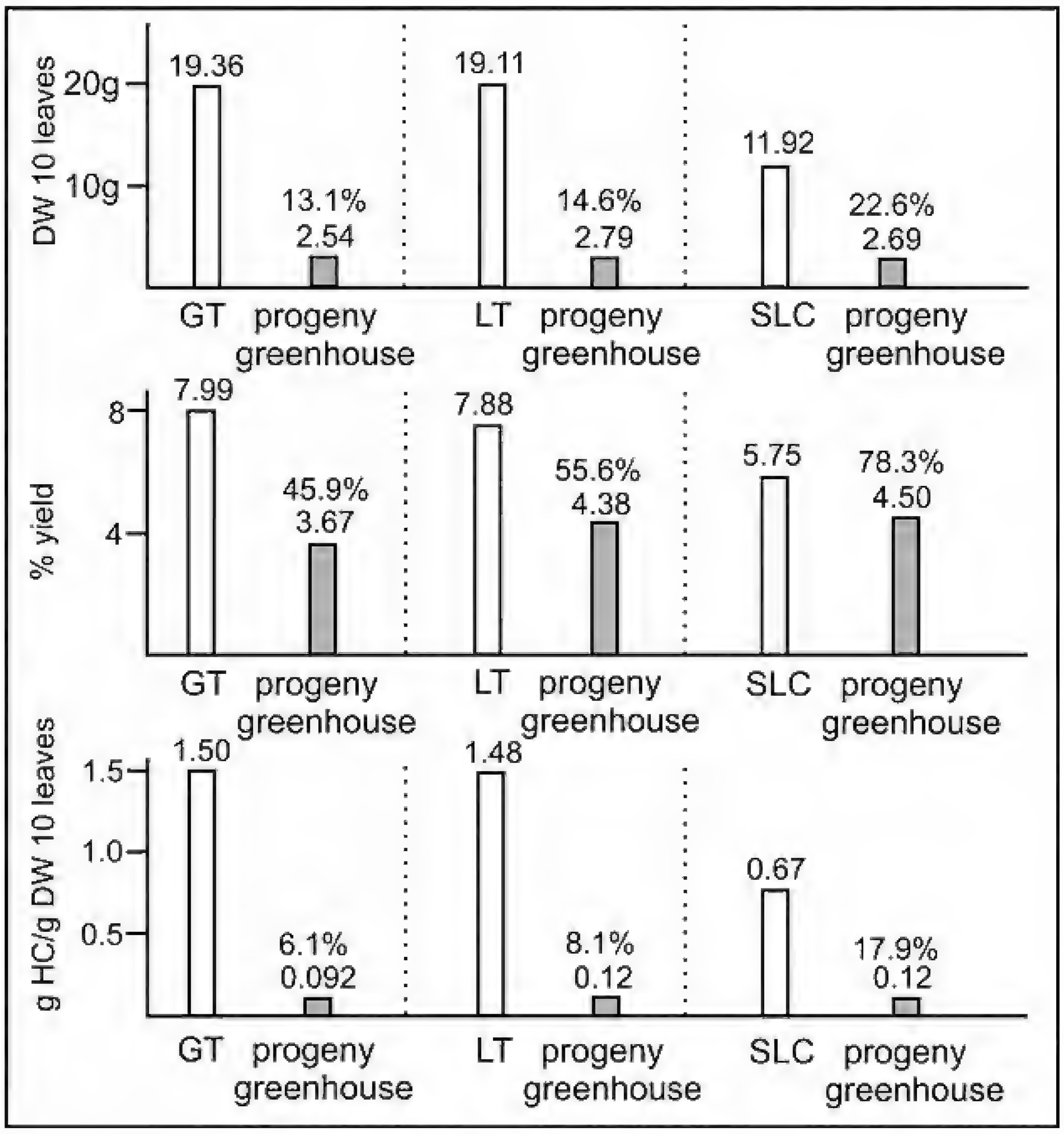

Figure 1. Comparison of DW 10 leaves, HC yield and g HC/ gDW 10 leaves for fields sampled sunflowers vs. their progeny grown in the greenhouse at OPSU (adapted from Adams et al. 2017c).

RESULTS

Table 1 presents the effects of MeJA on leaf biomass, % HC yields, and g HC/ g DW 10 leaves for cv. Little Becka. Each of these three variables were very highly significantly effected. Graphic analyses of these effects are shown in Fig. 2. Notice biomass, as estimated by dry weight of 10 leaves/ plant, increased in day 2 and again in day 4 samples (Fig. 2, solid line). The % HC yields declined from the control to 2d, further in 4d (Fig. 2, dotted line). HC yields, as g HC/ g DW 10 leaves, did not differ between control and d2, but did display a significant drop in d4 (Fig. 2, small dotted line). Punching 3 holes per leaf did not effect % HC or g HC, but did result in a significant decline in leaf biomass.

Table 1. Comparison of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Little Becka sprayed with 100 µM MeJA and analyzed after 2 and 4 days, plus plants sprayed and with leaves punctured with a hole punch, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05).

Little Becka component	control, no treatment	+ MeJA coll. 2 days later	+MeJA coll. 4 days later	+MeJA + holes in leaves, coll. 4 days later	F ratio, significance
wt. 10 leaves	1.72g^{b}	2.05g^{b}	2.76g^{a}	2.03g^{b}	F= 5.67 P = 0.47 x 10^{-2}***
% HC yield	3.31%^{d}	2.41%^{e}	0.92%^{f}	1.10%^{f}	F= 25.4 P=0.5 x 10^{-5} ***
g HC/ g 10 leaves	0.055g^{x}	0.051g^{x}	0.025g^{y}	0.022g^{y}	F= 10.6 P=0.25 x 10^{-3} ***

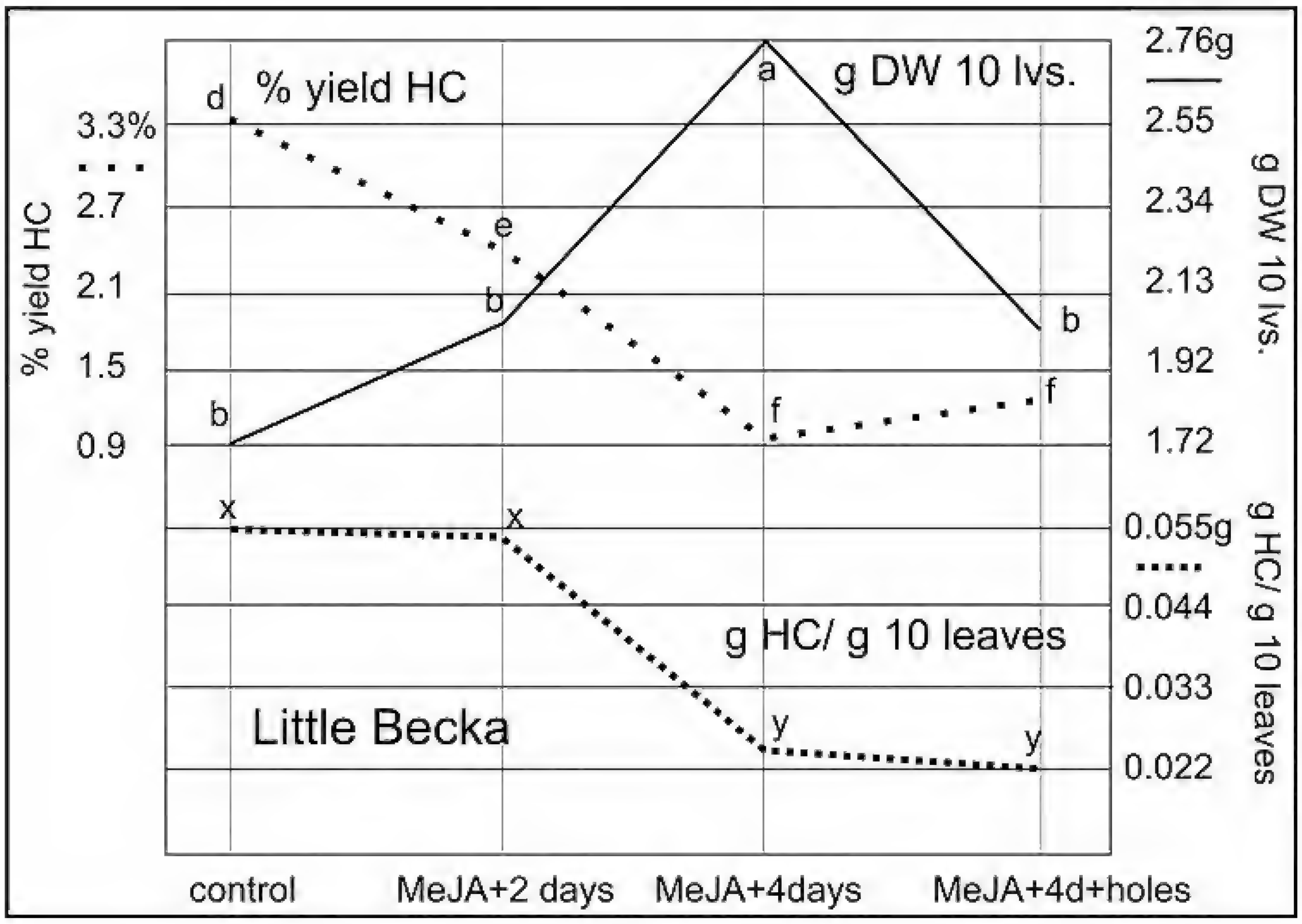

Figure 2. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Little Becka sprayed with 100 µM MeJA and analyzed after 2 and 4 days, plus plants with holes in the leaves and also sprayed with MeJA, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05). See text for discussion.

Table 2 presents the effects of MeJA on leaf biomass, % HC yields, and g HC/ g DW 10 leaves for cv. Munchkin. Each of these three variables were very highly significantly affected. Graphic analyses of these effects are shown in Fig. 3. Notice (Fig. 3) MeJA had no effects on leaf biomass for up to 7d, then a significant increase after 14d. It seems likely that this delayed growth was due to normal growth, after the MeJA had dissipated. The % HC yields declined after just 2d and remained low through day 7, then increased to, statistically, the same level as the control (Fig. 3). A similar pattern was seen (Fig. 3) in g HC/ g DW 10 leaves (Fig. 3).

Table 1. Comparison of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Little Becka sprayed with 100 µM MeJA and analyzed after 2 and 4 days, plus plants sprayed and with leaves punctured with a hole punch, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05).

Munchkin component	control, no treatment	+ MeJA coll. 2 days later	+MeJA coll. 4 days later	+MeJA coll. 7 days later	+MeJA coll. 14 days later	F ratio, significance
wt. 10 leaves	2.63g^{b}	2.36g^{b}	2.53g^{b}	2.46g^{b}	4.06g^{a}	F= 12.4 P = 0.41x10^{-4} ***
% HC yield	4.36%^{d}	3.32%^{ef}	3.26%^{ef}	2.99%^{f}	3.91%^{de}	F= 6.76 P=0.90 x 10^{-3} ***
g HC/ g 10 leaves	0.113g^{y}	0.077g^{z}	0.081g^{z}	0.073g^{z}	0.159g^{x}	F= 28.83 P=0.89 x 10^{-6} ***

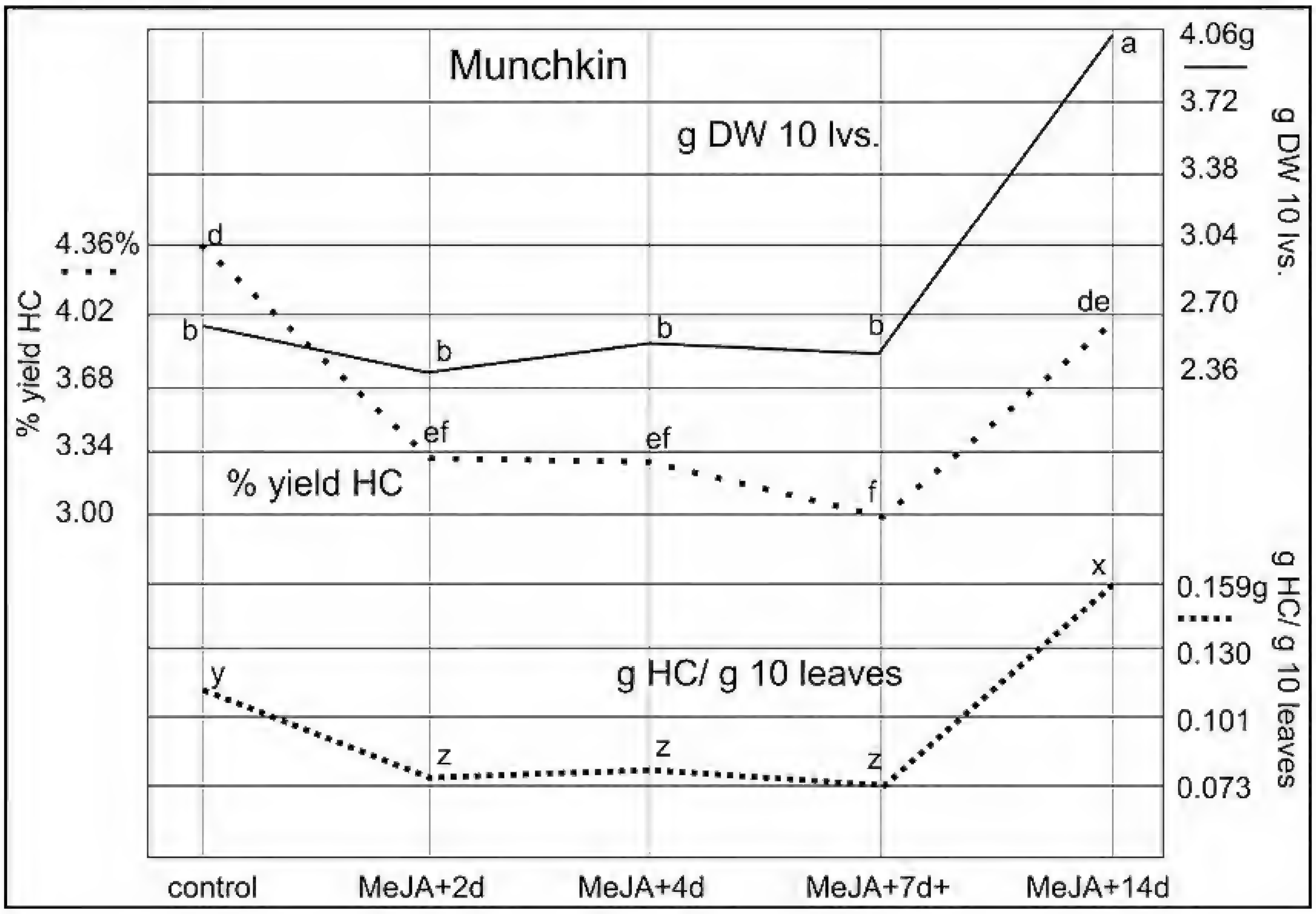

Figure 3. Graphs of dry weight (10 leaves), percent HC yields, and g HC/ gDW 10 leaves for Munchkin sprayed with 100 µM MeJA and analyzed after 2 and 4 days, plus plants with holes in the leaves and also sprayed with MeJA, then analyzed after 4 days. Means with a different letter superscripts are significantly different (P= 0.05). See text for discussion.

This research was initiated in the hope of stimulating the production of HC (as secondary compounds). However, although MeJA had significant effects on both Little Becka and Munchkin, unfortunately, it decreased the free HC by over 50% in 2 to 4 days. This seems to imply that the application of MeJA is not useful in increasing the free HC for alternative fuels from sunflowers.

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Feather-tipped Forceps: A method for handling fragile organisms

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ABSTRACT

Feather-tipped forceps are useful for transferring fragile objects. We have found feather-tipped forceps to practically eliminate specimen breakage and generally facilitate the handling of diverse structures such as nematode cysts and floral anthers. Feather tips allow for complete compression of the forceps eliminating the partial closure of forceps that is necessary when standard forceps are used to handle fragile objects. Partial closure of standard forceps can cause accidental release when too little pressure is applied, and destruction when too much pressure is applied. It is likely that feather-tipped forceps would be useful in diverse subdisciplines of biology wherever fragile parts of organisms are handled. We present the steps necessary for assembly of feather-tipped forceps because they are apparently not commercially available. Published on-line www.phytologia.org *Phytologia 100(3): 1-5 (Sep 21,, 2018).* ISSN 030319430.

KEY WORDS: anther, feather-tipped forceps, nematode cyst, transferring

Feather-tipped forceps (Figures 1 & 2) are useful for transferring small, fragile living or formerly living organisms or parts of organisms. Feather-tipped forceps are used in entomology for transferring bed bugs (Pfiester et al. 2008; Pereira et al. 2009; Lehnert et al. 2011) but neither a vendor nor instructions for construction are mentioned by these authors. Feather-tipped forceps are apparently not widely used in biology due to lack of awareness and / or lack of commercial availability. We wish to convey the utility of feather-tipped forceps for handling fragile structures, and present the steps necessary for assembly.

MATERIALS AND METHODS

How to make feather-tipped forceps. One of us (M.C.S.) modified off-the-shelf forceps by grinding the tips on a standard grinding wheel until the tip was blunt, but also began assembly with other stock models of forceps having blunt tips. The first cut in the feather was parallel to the rachis, near the midlength, and was about 8 mm long (Figure 1A). This section of vane, when removed, was ready to be fixed to the jaws of the forceps (Figure 1B). E6000® craft adhesive (available at stores such as Hobby Lobby, Michaels, WalMart, etc.) was applied to the inside of the forceps jaws one at a time (Figure 1C). Segments of feather 2 cm long by several mm wide were glued to the inner face of the jaws (distal ends) of forceps, one to each jaw, orienting the feather parallel to the length of the forceps (Figures 1D & E). The jaws of the forceps were then clamped together with a small binder clip until the glue dried, and then the clamp was removed. The feathers were easily trimmed longitudinally, starting at the end of the feather that was glued to the forceps. We then compressed the forceps and cut both feathers at the same time making the tips even (Figure 1F). After trimming, the portion of the feather that came in contact with the specimen (the working face) was 6 to 12 mm long beyond the forceps. Somewhat larger or smaller working faces may be necessary for other applications. In practice, either glue or tape was used to bind the feather segments to the metal, with tape allowing easier replacement of feathers when they became frayed over time.

Feathers found on the ground, likely those of a wild turkey (*Meleagris gallopavo*), were used but it is likely that stiff feathers of other species would be suitable. It is illegal for anyone to take, possess, import, export, transport, purchase, barter or offer for sale, any migratory bird or its parts (U.S. Fish &

Wildlife Service). However, the feathers of domesticated species are readily available for purchase online. Tail or wing feathers (rectrices or remiges) of large domesticated birds (e.g., poultry, ducks) are stiffer than feathers from other parts of the body or smaller birds, and would likely be useful (S. L. Halkin, personal communication).

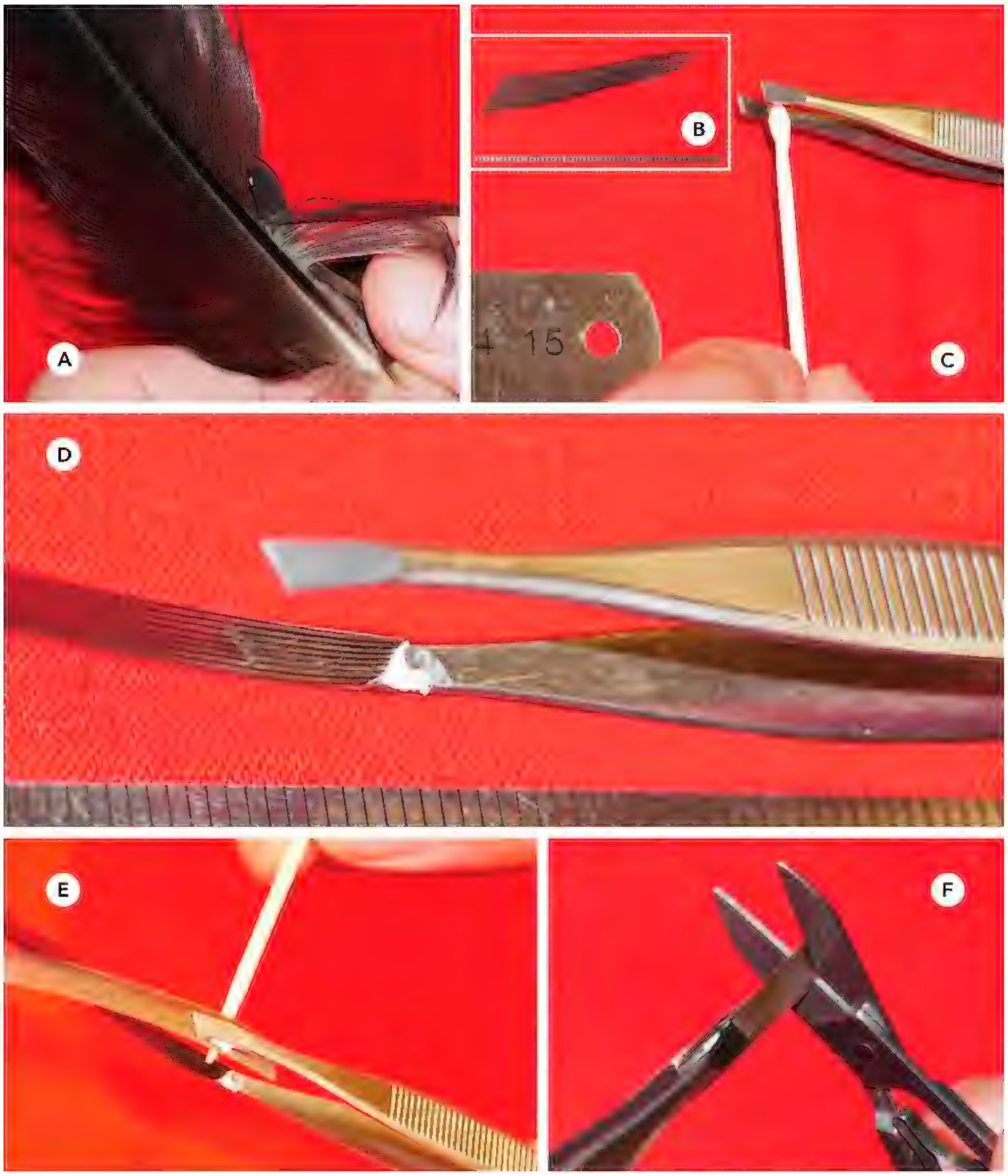

Figure 1. A–Feather was cut parallel to the rachis. B–Feather segment removed from the feather shown in A, units are mm. C–Glue was applied to jaws of forceps one side at a time, smallest units are mm. D–Feather was glued to one jaw of the forceps, units are mm. E–Feather was glued to the second jaw of the forceps. F–Forceps were compressed to cut both feathers at the same time, making the tips even. Photos by T. M.

RESULTS AND DISCUSSION

One challenge commonly encountered in biology is transferring small, fragile organisms without breaking or piercing the organism. In the past we transferred small organisms, or parts of organisms, by manually closing forceps with sufficient pressure to grasp the structure. The drawback of this method, of course, is that insufficient pressure results in accidental release and too much pressure results in destruction of the structure. Featherweight forceps allow improved handling of fragile organisms over standard forceps but lack feather tips and consequently one can crush fragile structures (featherweight forceps are available from BioQuip.com, among other vendors). In contrast, the use of feathers at the tips allows for complete compression of the forceps without crushing organisms. Feather-tipped forceps (Figure 2A), a modification of standard forceps, are inexpensive to produce, easy to use, and can be customized to meet specific requirements.

Pollen counts, the number of pollen grains produced by each flower, are useful in a variety of plant reproductive biology studies (Mione & Anderson 1992; Kearns & Inouye 1993; Mione et al. 2016; Mione et al. 2017). To estimate pollen production, anthers (prior to dehiscence) are softened in 1 N HCl (Anderson & Symon 1989). After softening but before immersion in the final solution in which pollen is counted, there is the real possibility that handling the anthers with standard forceps will result in breakage, releasing pollen, leading to an underestimate of pollen production. One of us (T.M.) found feather-tipped forceps to be remarkably useful for transferring anthers (Figure 2B) both prior to softening and after the anthers are softened and consequently are most fragile.

Desiccated leaf fragments are hard yet fragile and must be handled for extraction of nucleic acids, and feather-tipped forceps allows leaf fragments to be handled with minimal breakage.

In nematode biology it is standard procedure to screen (sieve) soil for nematodes (Boeri & Chung 2012). The remaining organic matter along with the cysts is sorted and nematodes are collected. Khan (2008) presents a detailed chapter describing methods of nematode sampling, storage, extraction from soil and extraction from plant tissue. He describes transferring individual nematodes with a "fine steel needle, bamboo splinter, eyebrow, eyelash hair or horse tail hair," but does not describe how to transfer small numbers of cysts to a vial. One of us (M.C.S.) found that feather-tip forceps in conjunction with a small spatula can be used to sift through organic matter under a microscope putting the cysts to one side of the dish. The feather tips can be used to sweep the cysts (Figure 2C) into a manageable pile. Feather-tip forceps, with the jaws decompressed, were held at about 45 degrees to the dish. With the feather tip tight

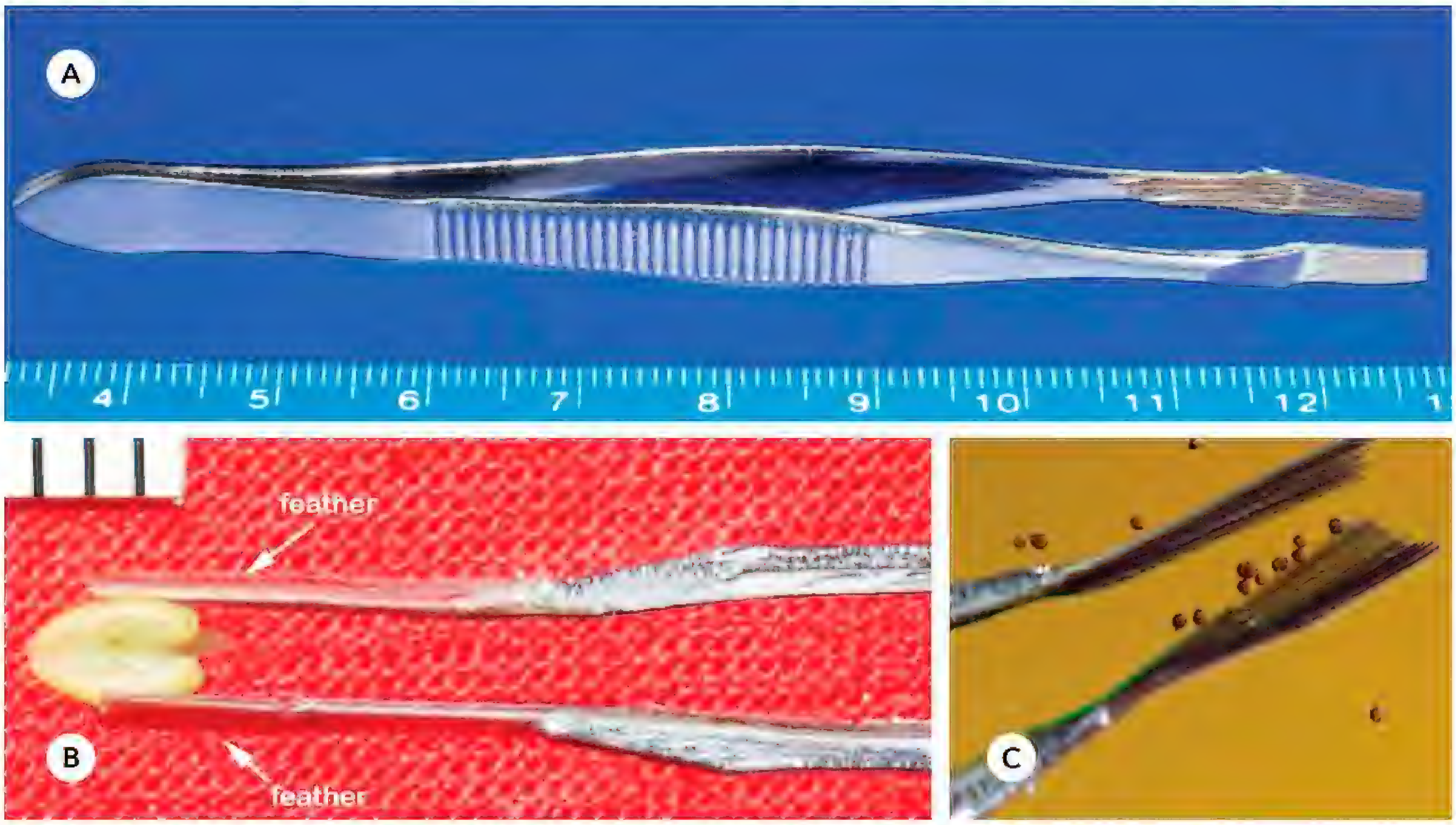

Figure 2. A–Feather tips (at right) were glued to stock forceps, numbered units are cm. B–Feather-tipped forceps holding anther, units in upper left are mm. C–Nematode cysts being transferred by feather-tipped forceps. Photos by M. C. S. & T. M.

to the dish the tips were completely compressed around the cysts. The feather tips did not harm the cysts. Once the cysts had been separated from the organic matter they were easily transferred into a vial by inserting the tips of the compressed forceps into the mouth of the vial and releasing pressure on the hinge. A gradual wipe against the rim (mouth) of the vial dislodged any cysts stuck to the feathers. When several cysts were to be transferred feather-tipped forceps were better than standard forceps because one can transfer multiple cysts with one sweeping motion and one compression of the forceps (Figure 2C).

CONCLUSIONS

Feather-tipped forceps are useful for handling delicate specimens because they eliminate the need to keep a constant pressure on the forceps. Feather segments were added to the tips of stock forceps (Figures 1 & 2). With this modification sorting and collecting nematode cysts under a dissecting microscope is efficient, simple, and safer for the cysts relative to use of traditional forceps. Feather-tipped forceps allowed transfer of anthers of flowering plants without puncture and allowed transfer of leaf fragments (that had been desiccated for later extraction of nucleic acids) without further fragmentation.

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Discovery of *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev in Albania and relictual polymorphisms found in nrDNA

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ABSTRACT

Additional analyses of trnS-trnG and nrDNA from specimens from Albania revealed the presence of *J. sabina* var. *balkanensis*. Careful chromatogram analysis of eight (8) polymorphic sites in nrDNA revealed that 6 of the 8 plants were polymorphic (RSRMYRK or RSRMARK) and apparently hybrids between type 1 GGACCCAG and type 2 (ACGATAGT) plants. Two plants were homozygous for the 8 sites and they both had the type 1 pattern (GGACCCAG). The type 2 pattern (ACGATAGT) was not found, but due to the presence of hybrids, it probably grows in the area. These two nrDNA types appear to have arisen via hybridization with a *J. thurifera* ancestor. The two types often are found in both v. *sabina* and v. *balkanensis* populations. Published on-line www.phytologia.org *Phytologia 100(2): 187-194 (Sep 22, 2018)*. ISSN 030319430.

KEY WORDS: *Juniperus sabina var. balkanensis, J. sabina*, distribution, nrDNA, trnS-trnG, chloroplast capture, ancient nrDNA heterozygotes.

Recently, Adams et al. (2018) reported on new populations of *J. sabina* var. *balkanensis* from Macedonia, Bosnia-Herzegovina, Croatia and central Italy (Fig. 1). As a part of this continuing study (Adams et al. 2016, 2017, 2018; Adams 2014), we now report on the confirmation of *J. sabina* var. *balkanensis* from Albania.

MATERIAL AND METHODS

Specimens used in this and previous studies: (species, popn. id., location, collection numbers): See Adams et al. (2018) for previously analyzed specimen locations.
Albania: North-Eastern Albanian Alps, above the road from Ceren to Radomira village, 41° 49' 43.86" N; 20° 27' 43.05" E. ca. 1150 m and above the village of Ceren, on path to Sorokol, 41° 49' 37.01" N; 20° 28' 28.13" E. ca. 1430 m**.** Coll. *Lulëzim Shuka s.n.* 22 June 2018. Lab Acc. *Adams 15506-15513* (8 samples). Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS primers utilized. The primers for trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. 2.31 (Technelysium Pty Ltd.).

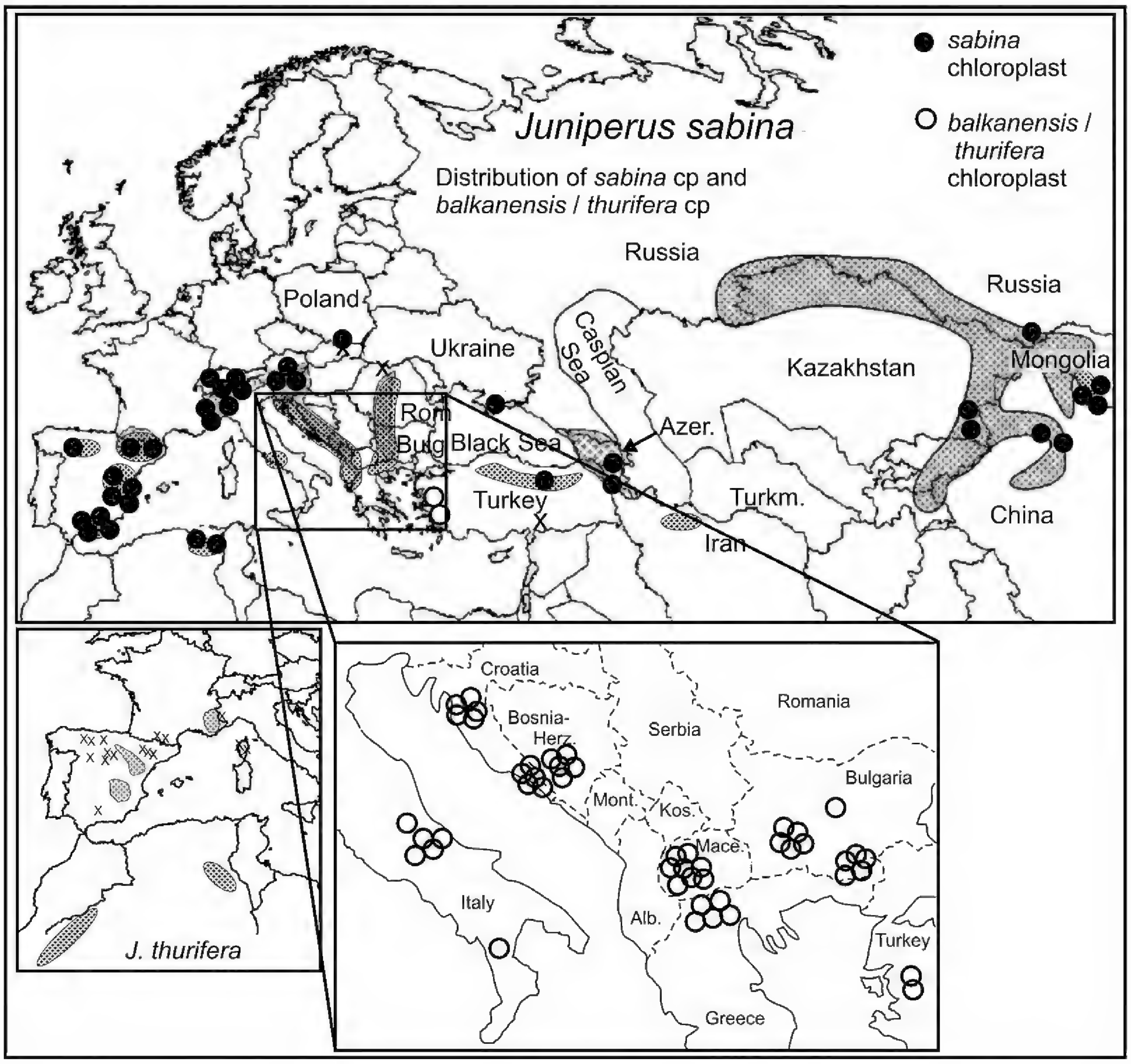

Figure 1. Distribution of *J. sabina* var. *balkanensis* and typical *J. sabina* chloroplast. The present day distributions of *J. thurifera* and var. *africana* (in north Africa) are shown in the insert on the lower left. (modified from Adams et al, 2018).

RESULTS

All 8 plants sampled in Albania were *J. s.* var. *balkanensis* based on cp DNA (Table 1). The revised distribution of v. *balkanensis* is shown in Figure 2. All known locations of v. *balkanensis* are in a relatively small geographical area.

Before discussing the nrDNA patterns, it should be noted that there are recent papers analyzing the inheritance of nrDNA in the Cupressaceae. Adams and Matsumoto (2016) analyzed 3 variable sites of nrDNA from synthetic crosses between *Cryptomeria japonica* cv. Haara and cv. Kumotooshi (= cv. Haara x Kumotooshi, ie., a backcross). They found that 3 of the 7 progeny had nrDNA very similar to that of the Haara x Kumo parent. In contrast, 4 of the 7 progeny had nrDNA exactly like the Haara parent. This appears to suggest that nrDNA polymorphisms can revert to that of a recurrent parent in the case of backcrossing.

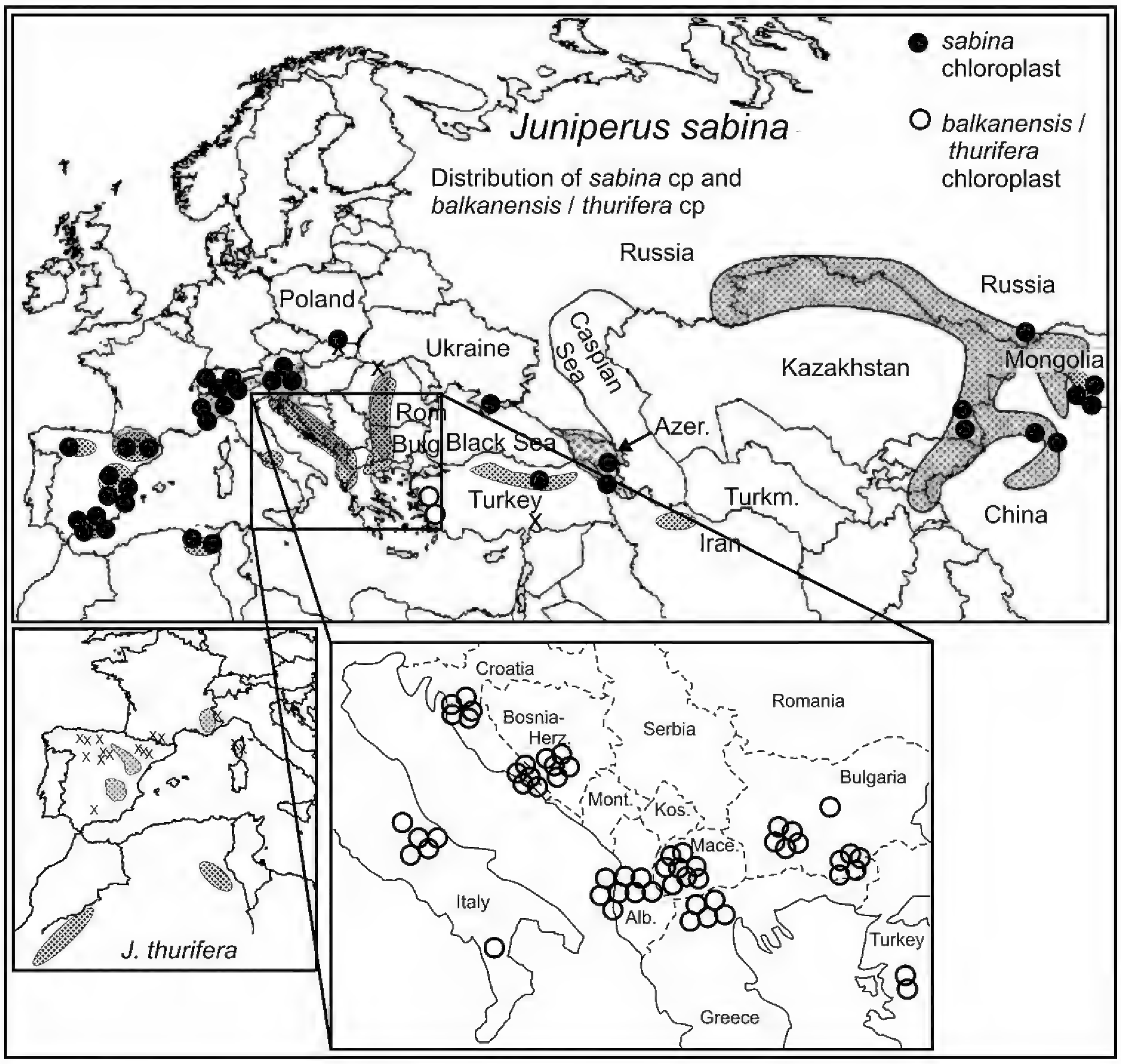

Figure 2. Revised distribution of *J. sabina var. sabina* (dark circles) and *J. s.* var. *balkanensis* (open circles) based on cp DNA (present study and from Adams et al. 2018)

Adams, Miller and Low (2016) examined 8 variable nrDNA sites in the parents (*Hesperocyparis arizonica*, *H. macrocarpa*), and their 18 artificial hybrid progeny. Each of the 18 hybrids were heterozygous for all 8 nrDNA sites. This study is very relevant to the present study, because *Hesperocyparis* (= *Cupressus* in the western hemisphere) is very closely related to *Juniperus* (Little et al. 2004, Terry, et al. 2012, Terry and Adams, 2015, Terry et al. 2016) and because there are no verified artificial hybrids of *Juniperus* available to the authors for the examination of the inheritance of nrDNA in *Juniperus*, the Adams, Miller and Low (2016) research on *Hesperocyparis* stands as a proxy for the inheritance of nrDNA in *Juniperus* and thus, their study in *Hesperocyparis* is surely applicable to *Juniperus*. So, we can confidently assume that nrDNA is inherited by complementation as found in *Hesperocyparis*, *Cryptomeria* and all other conifers. It should be noted that Adams, Miller and Low (2016) sequenced cp markers and confirmed that the cp genome is inherited via pollen (paternally inherited) in *Hesperocyparis* (and presumably *Juniperus*).

Aligning *J. sabina* and *J. thurifera* nrDNA sequences revealed that the taxa differ by SNPs at 22 sites, all in ITS1 or ITS2. Previously (Adams et al. 2018), a close examination of the nrDNA sequencing chromatograms revealed that generally, only 8 of the 22 sites contained heterozygous peaks. The 8 sites were (with position): 352(R), 391(S), 432(R), 606(M), 745(Y), 999(M), 1046(R), 1047(K). Two nrDNA types were found considering these 8 sites. Both of these types, type 1 (GGACCCAG) and type 2 (ACGATAGT), have been found in var. *balkanensis* and in var. *sabina* populations. Adams et al. (2018) reported the majority of the plants examined (42/62) were heterozygous for 1 to 8 sites, (Table 1). Note that 33/ 42 heterozygous individuals were heterozygous for all 8 sites. They reported the frequency of heterozygous sites was: 1,1,1,3,2,1,0,33 (for plants containing from 1 to 8 polymorphic sites, respectively).

In the Albania population, 2 plants (15508, 15511) were homozygous (for the 8 loci) and had the type 1 pattern: GGACCCAG. The other 6 plants were mostly heterozygous (Table 2). Of the 6 heterozygous plants, 15509 and 15510 were RSRYARK (7/8 heterozygous positions), and 15506, 15507, 15509, 15510, 15512, 15513 were RSRMYMRK (8/8 heterozygous positions) (Fig. 3). The type 2 ITS pattern (ACGATAGT, Adams et al. 2018) was not found among these 8 samples, but it surely must be present nearby, as the hybrids (between the 2 ITS types) were common.

Throughout the range of *J. sabina*, heterozygous plants are somewhat randomly distributed (Fig. 3). It is interesting that 4/4 Spain and 2/3 Switzerland plants were homozygous for the 8 sites. Only 2/7 plants from the far East were homozygous. Plants of var. *balkanensis* seem to be a bit more heterozygous (36/48 were heterozygous) than var. *sabina* (Fig. 3, Table 2).

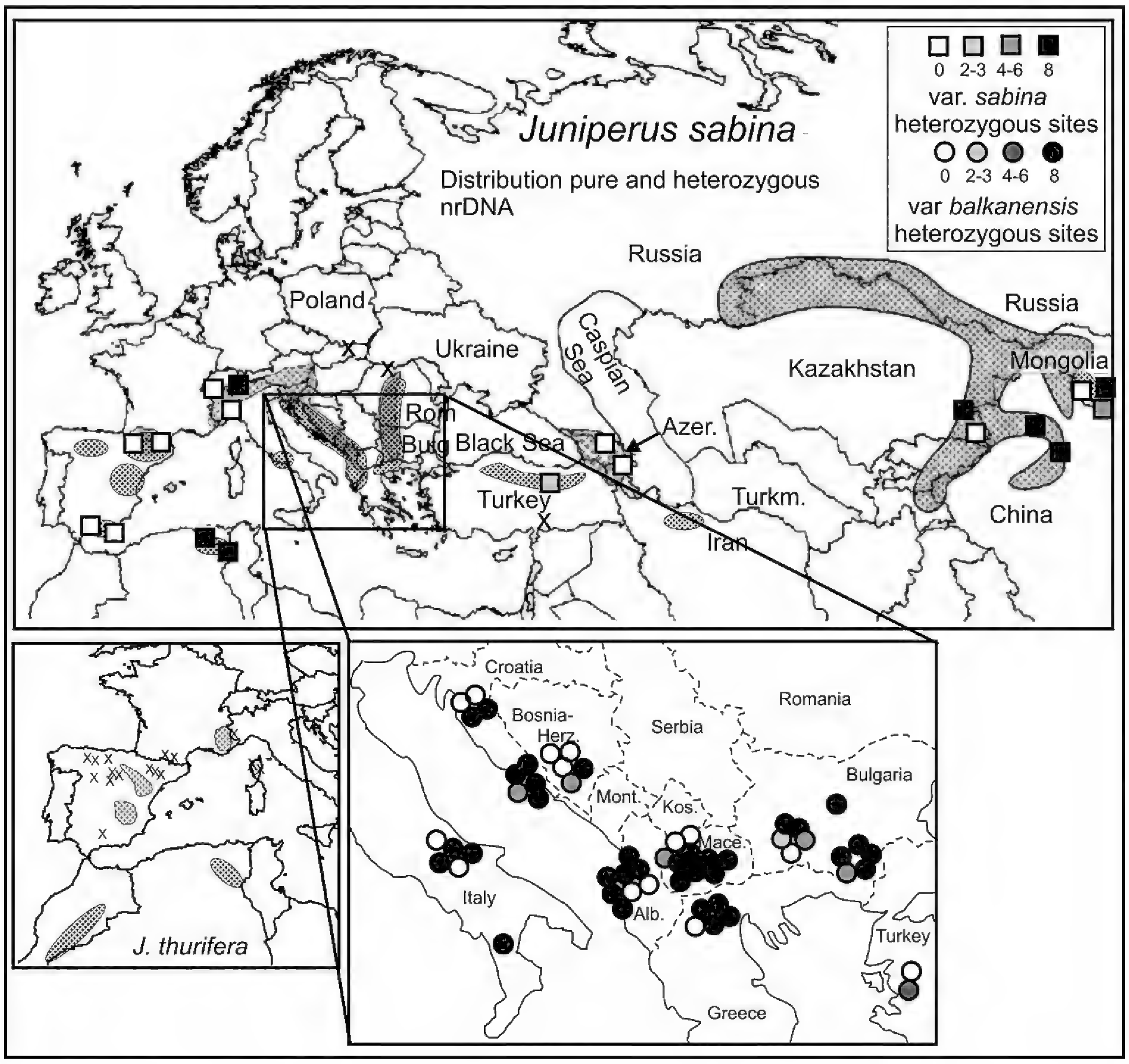

Figure 3. Distribution of homozygosity and heterozygosity among the 8 nrDNA polymorphic sites.

A more detailed examination of nrDNA type 1 (16/20) and type 2 (4/20) homozygous plants (Table 2) shows that both type 1 and type 2 plants were present in the Switzerland samples. One type 2 plant was found in Rila Mtn., Bulgaria and 2 were from Azerbaijan. All 16 type 1 nrDNAs share 4 bp sites with *J. thurifera*, and the four type 2 plants share 3 bp sites with *J. thurifera* (Table 2). It is strange that type 1 nrDNA contains 4 bp, typical of *J. thurifera* (in the 8 sites), and type 2 contain 3 different bp typical of *J. thurifera* (below and Table 2), but these are mutually exclusive in types 1 and 2.

most common sabina pattern (type 1)	G **G** A **C** *C* **C** A **G**	4 sites in common with *J. thurifera*
7083, thurifera, France, Morocco[2]	**A G G C T C G G**	
2nd common sabina pattern (type 2)	**A** C **G** A **T** A **G** T	4 sites in common with *J. thurifera*

Crossing type 1 (GGACCCAG) x type 2 (ACGATAGT) gives RSRMYMRK (see below), which is the same as the putative hybrids (below and Table 1) of RSRMYMRK. Our data indicates that crossing between types 1 and 2 nrDNA types seem common, 46/70 plants were RSRMYMRK. These two nrDNA types may have arisen via hybridization with a *J. thurifera* ancestor and subsequent backcrossing to *J. sabina*.

most common sabina pattern (type 1)	G G A C C C A G
2nd common sabina pattern (type 2)	A C G A T A G T
cross between *J. sabina* type1 x type2	R S R M Y M R K
Putative 'hybrid' pattern (table 1)	R S R M Y M R K

At our present level of understanding, the distributions of *J. s.* var. *balkanensis* and *J. thurifera* do not appear to overlap, negating modern hybridization. However, there were large changes in plant distributions in the Pleistocene and earlier, it seem probable that *J. thurifera*-like ancestors were sympatric with *J. sabina,* and presenting opportunities for chloroplast capture from *J. thurifera.*

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Table 1. Survey of *J. sabina* and classification based on ITS and trnS-trnG sequences. Putative hybrids and backcrosses [*J. sabina* x ancestor of *J. thurifera*] based on ITS polymorphisms are in **bold**.

coll. #, location	trnS-trnG classification (ie. cp genome)	ITS classif.	polymorphic sites[1] 1 2 3 4 5 6 7 8	ITS #poly/ 8 sites
14861 Spil Daği, Turk., Boratynski	**v. *balkanensis***	***sabina***	**R G R C Y C R G**	**4**
14934 Spil Dagi, Turkey, Mataraci	v. *balkanensis*	*sabina*	G G A C C C A G	0
13725 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**G G A M Y M R K**	**5**
13726 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13727 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13728 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
13729 eastern Rhodopes, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14721 Sokolna reserve, Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14722 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**G G A C Y C A G**	**1**
14723 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*	A C G A T A G T	0
14724 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**R G A C Y M A G**	**3**
14725 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14726 Rila Mtn., Bulgaria	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14727 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14728 Tsena Mtn., Greece	v. *balkanensis*	*sabina*	G G A C C C A G	0
14729 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14730 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
14731 Tsena Mtn., Greece	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15311 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15312 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15313 Mavrovo, Macedonia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15314 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15315 Mavrovo, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15316 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15317 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15318 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15319 Gaichnik, Macedonia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15320 Gaichnik, Macedonia	**v. *balkanensis***	***sabina***	**R C R A T M R K**	**5**
15277 Mt. Cabulja, Bosnia-Herze.	**v. *balkanensis***	***sabina***	**R S R A Y M R K**	**6**
15278 Mt. Cvsnica, Bosnia-Herze.	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**

15279 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	G G A C C C A G	0
15280 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	G G A C C C A G	0
15281 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	G G A C C C A G	0
15282 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15283 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**G G A C Y M R K**	**4**
15284 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15285 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15286 Mt. Biokovo, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15343 Velebit, Croatia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15344 Velebit, Croatia	v. *balkanensis*	*sabina*	G G A C C C A G	0
15345 Velebit, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15346 Velebit, Croatia	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15365 Calabria area, southern Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15413 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15414 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15415 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	G G A C C C A G	0
15416 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	G G A C C C A G	0
15414 Abruzzo area, central Italy	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15506 Ceren, Albania	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15507 Ceren, Albania	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15509 Ceren, Albania	**v. *balkanensis***	***sabina***	**R S R *A* Y M R K**	**7**
15510 Ceren, Albania	**v. *balkanensis***	***sabina***	**R S R *A* Y M R K**	**7**
15512 Ceren, Albania	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15513 Ceren, Albania	**v. *balkanensis***	***sabina***	**R S R M Y M R K**	**8**
15508 Ceren, Albania	**v. *balkanensis***	***sabina***	G G A C C C A G	0
15511 Ceren, Albania	**v. *balkanensis***	***sabina***	G G A C C C A G	0
7612 Switzerland	v. *sabina*	*sabina*	G C A C C C A G	0
7614 Switzerland	v. *sabina*	*sabina*	A C G A T A G T	0
14938 northeast Turkey Kandemir	**v. *sabina***	***sabina***	**R G R C T A G T**	**2**
14316 Azerbaijan	v. *sabina*	*sabina*	A C G A T A G T	0
14317 Azerbaijan	v. *sabina*	*sabina*	A C G A T A G T	0
7811 Kazakhstan, Paniflor	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7812 Kazakhstan, Paniflor	v. *sabina*	*sabina*	G G A C C C A G	0
7585 Mongolia, Altair Mtns.	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7586 Mongolia, Altair Mtns	**v. *sabina***	***sabina***	**A C G A Y M R K**	**4**
7587 Mongolia, Altair Mtns	v. *sabina*	*sabina*	G G A C C C A G	0
7836 China, Heaven Lake, Xinjiang	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
7837 China, Heaven Lake, Xinjiang	**v. *sabina***	***sabina***	**R S R M Y M R K**	**8**
most common sabina pattern	Type 1		G **G** A **C** C **C** A **G**	Type 1
7083, thurifera, France[2]			A **G** G **C** T C G G	0
9420, thurifera v. africana, Morocco[2]			A G **G** **C** T C G G	0
2nd most common sabina pattern	Type 2		**A** C **G** A **T** A **G** T	Type 2

[1] Eight polymorphic sites (1-8): R352, S391, R432, M606, Y745, M999, R1046, K1047.
350 xTGTCGGAG; 391 xGAGGTCCG; 432 xTCGTGTGC; 606 CGACAAGAx;
745(105) xCCAAAAGA; 999(333) xGCGAGGAG; 1046(392) xNGCGGTCGG;1047 xGCGGTCGG

[2] This pattern, **A G G C *T* C G G,** was also found in **ALL** *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, et al. 2018)

Table 2. *Juniperus sabina* classified based on ITS homozygous for all 8 polymorphic sites. Putative hybrids (heterozygous for the 8 polymorphic sites) were excluded.

coll. #, location	trnS-trnG classification	ITS classif.	nrDNA type	polymorphic sites[1] 1 2 3 4 5 6 7 8	# sites in common with *J. thurifera*
14934 Spil Dagi, Turkey, Mataraci	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
14728 Tsena Mtn., Greece	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15313 Mavrovo, Macedonia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15319 Gaichnik, Macedonia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15279 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15280 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15281 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15343 Velebit, Croatia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15344 Velebit, Croatia	v. *balkanensis*	*sabina*	1	G G A C C C A G	4
15508 Ceren, Albania	**v. *balkanensis***	***sabina***	**1**	**G G A C C C A G**	**4**
15508 Ceren, Albania	**v. *balkanensis***	***sabina***	**1**	**G G A C C C A G**	**4**
7197 Sierra Nevada, Granada, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7199 Sierra Nevada, Granada, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7573 Sallent deGallego, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7574 Sallent deGallego, Spain	v. *sabina*	*sabina*	1	G G A C C C A G	4
7812 Kazakhstan, Paniflor	v. *sabina*	*sabina*	1	G G A C C C A G	4
7587 Mongolia, Altair Mtns	v. *sabina*	*sabina*	1	G G A C C C A G	4,
7612 Switzerland	**v. *sabina***	***sabina***	**1**	**G G A C C C A G**	**4 Note Type 1!**
7614 Switzerland	**v. *sabina***	***sabina***	**2**	**A C G A T A G T**	**3, Note Type 2!**
14723 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*	2	A C G A T A G T	3
14316 Azerbaijan	v. *sabina*	*sabina*	2	A C G A T A G T	3
14317 Azerbaijan	v. *sabina*	*sabina*	2	A C G A T A G T	3
				bases in common (**bold**) w *thruifera*	
most common sabina pattern(type 1)			1	G **G** A **C** C **C** A **G**	4 sites in common
7083, thurifera, France, Morocco[2]				**A G G C T C G G**	8
2nd common sabina pattern(type 2)			2	**A** C **G** A **T** A **G** T	4 sites in common
14861 Spil Daği, Turk., Boratynski	v. *balkanensis*	*sabina*		R G R C Y C R G	4
13725 eastern Rhodopes, Bulgaria	v. *balkanensis*	*sabina*		G G A M Y M R K	5
14722 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*		G G A C Y C A G	1
14724 Rila Mtn., Bulgaria	v. *balkanensis*	*sabina*		R G A C Y M A G	3
15320 Gaichnik, Macedonia	v. *balkanensis*	*sabina*		R C R A T M R K	5
15277 Mt. Cabulja, Bosnia-Herze.	v. *balkanensis*	*sabina*		R S R A Y M R K	6
15283 Mt. Biokovo, Croatia	v. *balkanensis*	*sabina*		G G A C Y M R K	4
14938 northeast Turkey Kandemir	v. *sabina*	*sabina*		R G R C T A G T	2
7586 Mongolia, Altair Mtns	v. *sabina*	*sabina*		A C G A Y M R K	4
most common sabina pattern)			Type 1	G G A C C C A G	
2nd common sabina pattern			Type 2	A C G A T A G T	
cross between *J. sabina* type1 x type2			1x2	R S R M Y M R K	
Putative 'hybrid' pattern (table 1)				R S R M Y M R K	

[1] Eight polymorphic sites (1-8): R352, S391, R432, M606, Y745, M999, R1046, K1047.

[2] This pattern, AGGCTCGG**,** was found in all *J. thurifera* samples examined, to date (14 *J. thurifera* samples from Corse, Morocco, France and Spain, Adams, et al. 2018)

The effects of growth regulators on the growth and yields of hydrocarbons in *Cotton* (*Gossypium hirsutum*)

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ABSTRACT

Glyphosate, 2,4-D, methyl jasmonate, and ethephon, sprayed on leaves of cotton (SA-2269), produced non-significant changes in leaf biomass. These treatments also resulted in lower % HC yields in the order of: control (not sprayed), glyphosate, 2,4-D, methyl jasmonate, 100 ppm Florel and 1000 ppm ethephon. No significant differences were found in g HC/ g DW 10 leaves. Published on-line www.phytologia.org *Phytologia 100(4): 195-198 (Dec 21, 2018).* ISSN 030319430.

KEY WORDS: Cotton, *Gossypium hirsutum*, yields of hexane extractable leaf hydrocarbons, growth regulators, 2,4-D, glyphosate, methyl jasmonate, ethephon.

Cotton (*Gossypium* sp.) is a subtropical, perennial, woody plant and, as such, has an indeterminate growth pattern. However, in most of the world it is grown as a short life cycle "annual". Excessive vegetative growth can be detrimental as fruit may abort, and crop maturity can be delayed leading to a reduced harvest (Jost, et al. 2006).

Opitz, Kunert and Gershenzon (2008) examined the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They found that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments. In addition, they reported that herbivory or mechanical damage in older leaves led to terpenoid increases in younger leaves. Higher terpenoid yields were achieved by two methods: 1. increased filling of existing glands and 2. the production of additional glands. The composition of the terpenoid mixture did not significantly differ in response to herbivore, mechanical damage or jasmonic acid treatments.

Many plant species protect themselves from herbivory by a response to an attack (see Karban and Myers, 1989 for a review). Early research on plant defensive chemicals focused on constitutive (or stored) chemicals such as terpenoids, tannins and aromatic metabolic compounds derived from the shikimic acid pathway (Pare and Tumlinson, 1998). But, more recently, greater focus has been on inducible plant defenses (Chen 2008; Pare and Tumlinson, 1997, 1998; Turlings, et al. 1995). Turlings et al. (1995) published a seminal paper entitled "How caterpillar-damaged plants protect themselves by attracting parasitic wasps". They showed that plants injured by herbivores emit chemical signals that attract and guide the herbivores' natural enemies to the damaged plants. Thus, indirectly, injured plants send out a "SOS" signal for help against herbivores. Pare and Tumlinson (1997) nicely documented this phenomenon in a series of experiments on cotton using beet army worms and mechanical damage to leaves.

Chen (2008) discusses that some constitutive chemicals may be increased to even higher levels after insect attack. The present research (herein) is concerned with total extractable hydrocarbons for alternative fuels and chemical feedstocks from cotton leaves.

Plant Growth Regulators (PGRs or GRs) are very widely used in the cultivation of cotton (Rademacher 2015; Jost et al. 2006; Dodds, et al. 2010). PGRs are applied to balance vegetative and reproductive growth. PGRs are used to control excessive vegetative growth (PIX, mepiquat chloride; mepiquat pentaborate; cyclanilide, Stance® (cyclanilide+mepiquat chloride), etc., Rademacher 2015). PIX works by inhibiting cyclases (Rademacher 2015) involved in the synthesis of gibberellic acid (GA), thence leading to loss (decrease) of GA based compounds (eg. GA3, GA4, etc.). GAs promote longitudinal growth in plants (among other factors) (Rademacher 2015). Cyclanilide inhibits transport of natural auxins, and thus, reduces growth in cotton (Rademacher 2015).

Recently, Adams and TeBeest (2018) reported on the effects of a growth regulator (Stance®) on biomass, % HC yields and g HC/ g leaf biomass in commercial cotton. They reported significant differences were not found in leaf biomass or HC yield (as g/ weight 10 DW leaves). However, there was a significant difference (p= 0.05) in % HC yields. Plants sprayed with the growth regulator had a lower % HC yield. In contrast, plants not sprayed had a higher % HC yield. The use of a foliar spray containing both mepiquat chloride and cyclanilide (Stance®) resulted in the production decreased amounts of stored HC in cotton. It appears that Stance® not only disrupts gibberellic acid synthesis and the transport of auxins, but likely influences other synthesis pathways (including those leading to stored hydrocarbons).

This study examined the effects of foliar applications of glyphosate (ex RM43®, 43.68% glyphosate), 2,4-D (Fertilome Weed-Out®, (6.42%, 2,4-D, dimethylamine salt; 2.13% Qinclorac; 0.6% Dicamba, dimethyl amine salt), methyl jasmonate, 100 ppm and 1000 ppm ethephon (Florel® , 3.9% ethephon) on growth and HC yields in cotton (accession SA-2269).

MATERIALS AND METHODS

Plant Materials:

Cotton, SA-2269, was grown in Hurricane, UT, at Connie Stratton's garden plot (37° 09' 34.59" N, 113° 19' 36.63" W, elev. 3265 ft., sandy-loam, soil, watered at needed). An individual plant was bagged with large plastic bags (to prevent spray from drifting onto other plants) hand sprayed until spray wetted and coated each leaf. Five plants in each treatment were sprayed with:

1. 1000 ppm, glyphosate [ex RM43®, 43.68% glyphosate (cf. Roundup®)]. Ragan and Massey, Inc., Ponchatoula, LA
2. 100 ppm, 2,4-D (ex Fertilome Weed-Out®, 6.42%, 2,4-D, dimethylamine salt; 2.13% Qinclorac; 0.6% Dicamba, dimethyl amine salt), VGP, Bonham, TX
3. 100 µM, methyl jasmonate, Sigma-Aldrich, St. Louis, MO., USA
4. 100 ppm, ethephon (ex Florel® , 3.9% ethephon [2-chloroethyl phosphoric acid]), Lawn and Garden Products, Fresno, CA.
5. 1000 ppm, ethephon (ex Florel® , 3.9% ethephon [2-chloroethyl phosphoric acid]), Lawn and Garden Products, Fresno, CA.

The ten (10) lowest growing, non-yellowed leaves were collected and air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). Three gram aliquot of air dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. The soxhlet correction factor (sCF) was determined to be 1.14. All shaker extraction yields were corrected to oven dry weight (ODW) by multiplication of 1.085. Thus, the total CF was 1.24 (1.14 x 1.08). ANOVA and SNK (Student-Newman-Keuls multi-range tests) were performed in program SNK (by RPA) as formulated in Steel and Torrie (1960).

RESULTS

Biomass and hydrocarbon (HC) yields for control (not sprayed) and plants sprayed with various Growth Regulators (GR) are given in Table 1. A significantly (P=0.05) larger g DW/ 10 leaves was found in plants sprayed with 1000 ppm ethephon. Percent (%) HC yields, was negatively effected by all GRs. Yields were highest in the control plants, then decline to the lowest yields in 1000 ppm ethephon. Yields based on g HC/ g DW 10 leaves had no significant differences (Table 1).

Table 1. Comparison of leaf biomass and HC yields for cotton (SA-2269) sampled 4 days after spraying with a growth regulator. Any data values that share the same superscript are not significantly differ at P=0.05 by SNK multi-range tests. F (from ANOVA) significance: **=0.01. ns = non-significant (at P = 0.05)

SA-2269, treatment	g DW/ 10 leaves	% HC yield	g HC/ g DW 10 leaves
Control	3.78g^{B}	4.77%^{A}	0.152g^{A}
1000 ppm glyphosate	5.45g^{AB}	4.24%^{AB}	0.234g^{A}
100 ppm 2,4-D	4.23g^{AB}	4.19%^{AB}	0.174g^{A}
100 µM methyl jasmonate	5.16g^{AB}	3.72%^{B}	0.191g^{A}
100 ppm ethephon	4.28g^{AB}	3.42%^{B}	0.171g^{A}
1000 ppm ethephon	5.56g^{A}	3.40%^{B}	0.191g^{A}
F, Significance.	F=2.84 P=0.035 ns	F=4.04 P=0.008**	F= 1.21 P=0.334 ns

This study found that spraying cotton (SA-2269) with glyphosate, 2,4-D, methyl jasmonate, and ethephon resulted in non-significant changes in leaf biomass (although, all treatments resulted in a slightly higher leaf biomass, than in the control). These treatments resulted in lower % HC yields and no significant differences in g HC/ g DW 10 leaves.

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Comparison of hydrocarbon yields in SA-2269 cotton grown in four test plots in Texas and Utah

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ABSTRACT

Cotton accession, SA-2269, was grown in test plots at College Station, TX, Lubbock, TX, Oslo, TX and Hurricane, UT in 2017 to compare the environmental effects on leaf biomass, % yield of hydrocarbons (HC), and total HC (g HC /g leaves) under natural growth conditions. Very highly significant differences in g dry weight (DW) 10 leaves, % yield HC and g HC/ g DW 10 leaves were found among the test plots. Leaf biomass and g HC/ g DM 10 leaves declined as expected in the drier plots. However, the arid plot at Hurricane, UT, in the northeast Mojave Desert, had significantly larger % yield HC (8.03%), supporting the theory that drought stress can induce the synthesis of chemicals in cotton. Published on-line www.phytologia.org *Phytologia 100(4): 199-205 (Dec 21, 2018)*. ISSN 030319430.

KEY WORDS: Cotton, *Gossypium* spp., yields of hexane extractable leaf hydrocarbons, petrochemicals, liquid fuels.

During routine screening of cotton accessions (Adams et al. 2017a), five high HC yielding accessions were discovered in the summer of 2016 in a seed production plot at USDA, College Station, TX (SA-1166, 13.73%; SA-1419, 13.23%; SA-1181, 12.32%; SA-3348, 11.34%; SA-2269, 11.09%).

To analyze genetic relationships among these accessions, 597 SSR bands (Hinze et al., 2016) from the 30 accessions screened for HC were used in Principal Coordinate Analysis (PCoA). The analysis showed the accessions were divided into *G. barbadense* and *G. hirsutum* (Fig. 1, left and right). The *G. barbadense* samples (8) are all improved accessions. The samples of *G. hirsutum* contain both wild and improved accessions forming a very loose group, but the wild accessions are mostly found in the upper-right quadrant of the ordination (Fig. 1). Mapping the high HC yielding accessions onto the PCoA ordination (Fig. 1) reveals they are clearly clustered in a tightly grouped set of improved accessions (Fig.

1, dashed oval). Plotting the high and highest yielding samples revealed that the three samples representing the top 13% in HC yield (SA-1181, SA-1403, SA-2269) and the highest yielding sample (SA-1419, top 3%) are found in that group (Fig. 1, dashed oval). Adams et al. (2017a) noted that the discovery of the highest yielding samples in a group of improved accessions was surprising, in view of the selection for increased cotton seed and fiber yields. Accessions selected for improved agronomic yield were not expected to also have increased HC yields.

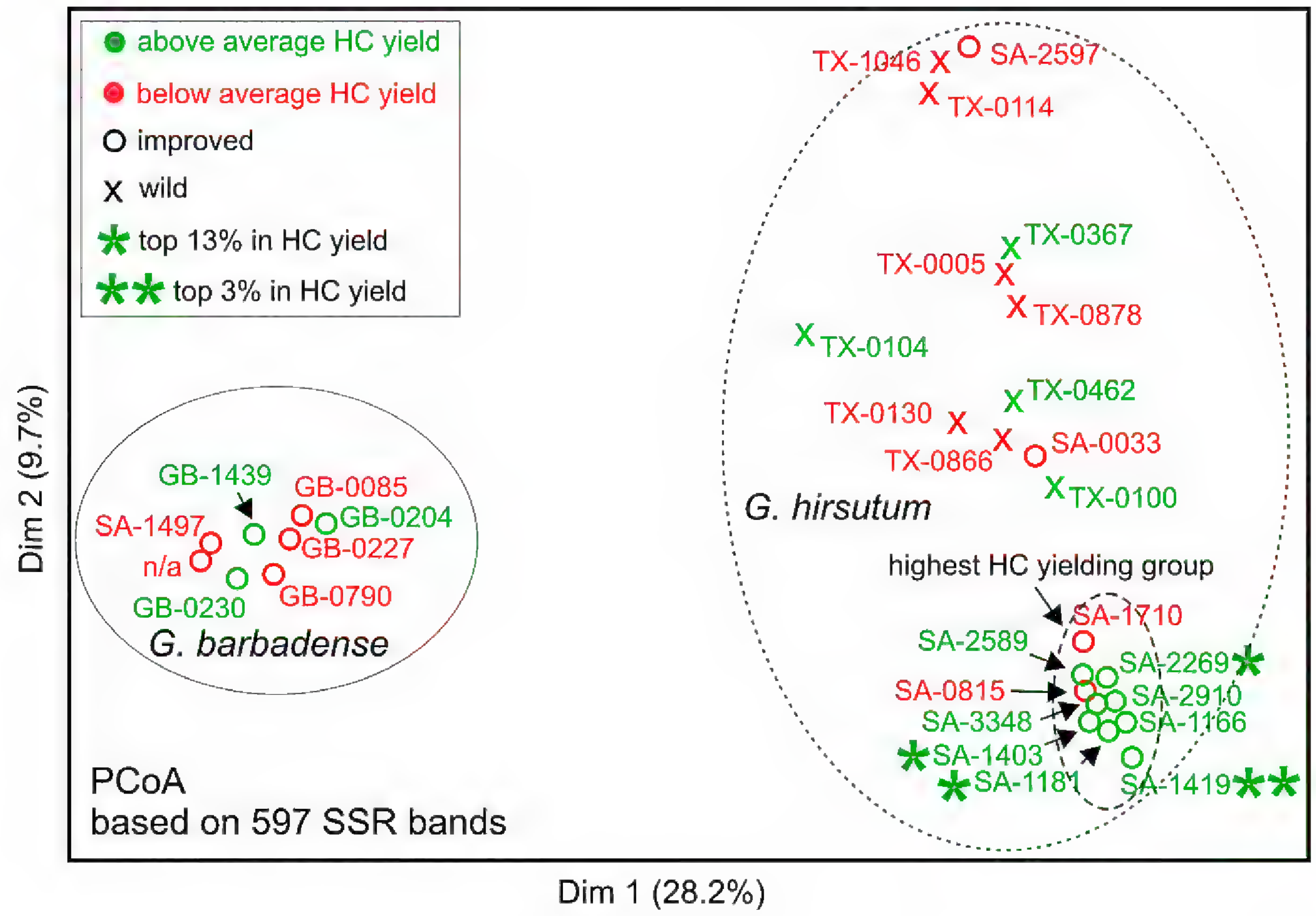

Figure 1. Principal Coordinate Analysis (PCoA) based on 597 SSR bands. The percent of variance accounted for among accessions is given on Dim 1 and Dim 2. From Adams et al. (2017a).

Adams et al. (2018a) expanded the survey by analyzing 26 additional accessions chosen from those that clustered nearest the "highest HC yielding" group (see Hinze et al. 2016). Surprisingly, %HC yields for nearly all 26 accessions (bottom, Fig. 2) had lower yields (<7.35%) than all but 7 of the 30 accessions sampled in 2016 (top, Fig. 2). It seems unlikely that more high yielding accessions were not discovered.

The question 'Are the high HC yields genetic or environmentally controlled?' led to follow-up studies (Adams et al. 2017a, b, 2018b) in which SA-2269 was grown in various environmental conditions. Table 1 shows that dry leaf weights were similar in all the situations. Percent HC yield was very high (11.09%) at College Station in 2016, but in the next test plot (2017), it dropped to 5.97%, and lower in greenhouse plants, 4.5%. g HC/ g 10 leaves mirrored the % HC yield, being nearly twice as high as when grown in other environments.

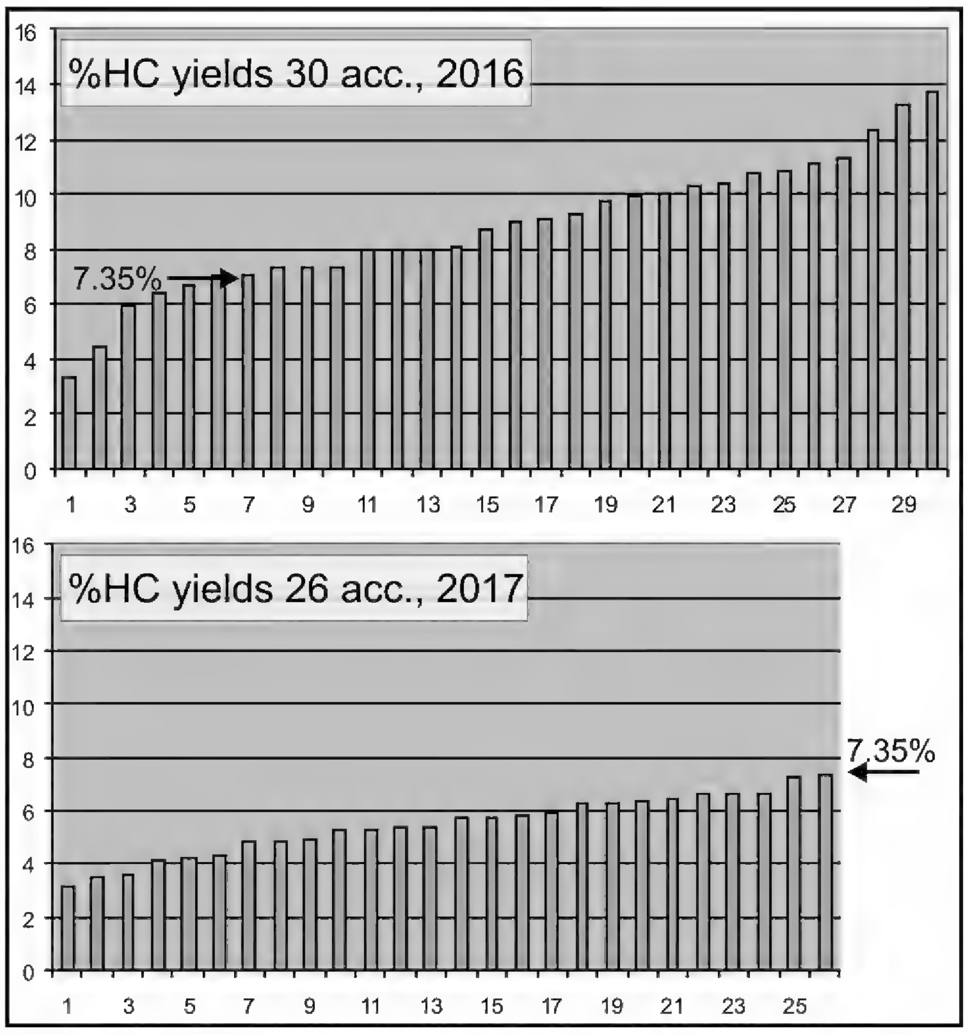

Figure 2. Histograms of % HC yields: 30 acc. (2016) (top); and 26 acc. (2017) (bottom).

Table 1. Comparison % HC yields, and g HC/ g DW 10 leaves from accession SA-2269 grown under ambient conditions at College Station, TX (2016, 2017), and grown in the greenhouse, Lubbock, TX (data from Adams et al. 2017a,b, 2018).

SA-2269, variable	Field, College Station, TX, 2016	Field, College Station, TX, 2017	Greenhouse, Lubbock, TX, 2016-2107
wt. 10 leaves	12.44	13.61	11.93
% HC yield	11.09%	5.97%	4.5%
g HC/ g DW 10 leaves	1.38	0.82	0.537

It seems that an unusual environmental incident occurred in the test plot at College Station in 2016. In a seminal paper, Stipanovic, Bell and Benedict (1999) reviewed the defensive role of pigment gland constituents in cotton. Cotton gland constituents (sesquiterpenoids, gossypol, and gossypol derivates, etc.) are a constitutive defense resource for cotton resistance to insects and diseases. Stipanovic, Bell and Benedict (1999) also discussed that these gland constituents can be rapidly synthesized in response to pathogens. Chen (2008) discusses that some constitutive chemicals may be increased to even higher levels after insect attack.

Opitz, Kunert and Gershenzon (2008) examined the response of stored (constitutive) terpenoids in cotton subjected to mechanical damage, herbivory and jasmonic acid treatments. They found that terpenoid levels increased successively from control to mechanical damage, herbivory, and jasmonic acid treatments.

The purpose of this paper is to report on changes in HC production in field cultivated cotton grown under ambient conditions in four environments: College Station, TX; Lubbock, TX; Oslo, TX and Hurricane, UT.

MATERIALS AND METHODS

Plant Materials:
Accession SA-2269 from the U.S. National Cotton Germplasm Collection.

2017 Environments:
College Station, TX
Cultivated at the USDA-ARS Southern Plains Agricultural Research Center, College Station, TX, 30° 37' 5.00" N, 96° 21' 50" W, 354 ft., subsurface drip irrigation, sandy soil, annual rainfall 40". The lowest growing, non-yellowed, mature leaf was collected at random, from each of 10 cotton plants.

Lubbock, TX
Cultivated at the USDA-ARS Plant Stress and Germplasm Development Research Center, Lubbock, TX, 33° 35' 36.3" N, 101° 54' 4.2" W, 3243 ft., light, sandy soil, avg. annual rainfall 19.2", water was applied during the growing season to attain germination and limited growth to reflect plant water stress responses, similar to dryland production, otherwise the plants were watered only by natural rainfall. The lowest growing, non-yellowed, mature leaf was collected at random, from each of 10 cotton plants.

Hurricane, UT
Cultivated in a garden plot on the Scow Family Farm, Hurricane, UT, 37° 10' 10.72" N, 113° 19' 14.0" W, 3277 ft., sandy, gravely soil, avg. annual rainfall 12-16". The ten (10) lowest growing, non-yellowed mature leaves were collected at random from each of 10 cotton plants, at 1st open flower stage.

Oslo, TX
Cultivated in garden plot, JP TeBeest Farm, 36° 25' 0.6" N, 101° 32' 17.3" W, 3258 ft., on dryland, dark, loam soil, Oslo, TX, avg. annual rainfall, 19.3". The ten (10) lowest growing, non-yellowed mature leaves were collected at random from each of 10 cotton plants, at 1st open flower stage.

Processing Leaf Samples:

Leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained. Leaves were ground in a coffee mill (1mm). Three grams of air-dried material (7% moisture) was placed in a 125 ml, screw cap jar with 20 ml hexane, the jar sealed, then placed on an orbital shaker for 18 hr. The hexane soluble extract was decanted through a Whatman paper filter into a pre-weighed aluminum pan and the hexane evaporated on a hot plate (50°C) in a hood. The pan with hydrocarbon extract was weighed and tared.

The shaker-hexane extracted HC yields are not as efficient as soxhlet extraction, but much faster to accomplish. To correct the hexane yields to soxhlet yields, one sample was extracted in triplicate by soxhlet with hexane for 8 hrs. The soxhlet correction factor (sCF) was determined to be 1.14. All shaker extraction yields were corrected to oven dry weight (ODW) by multiplication of 1.085. Thus, the total CF was 1.24 (1.14 x 1.08).

ANOVA and SNK (Student-Newman-Keuls multi-range tests) were performed in program SNK (by RPA) as formulated in Steel and Torrie (1960).

RESULTS

Biomass, hydrocarbon (HC) yields, g HC/ g 10 leaves from SA-2269 plants grown at College Station, TX; Lubbock; TX; Oslo, TX and Hurricane, UT are shown in Table 2.

Table 2. Leaf biomass, hexane extractable hydrocarbon (HC) yields, and g HC/ g DW 10 leaves from accession SA-2269 grown in the summer, 2017 at College Station, TX, Lubbock, TX, Oslo, TX and Hurricane, UT. Any data values that share the same superscript are not significantly different at P=0.05 by SNK multi-range tests. Significance: *=0.05; **=0.01; ***>0.001.

SA-2269 grown in 2017	College Station, TX	Lubbock, TX	Oslo, TX	Hurricane, UT	F, Significance.
g DW 10 leaves	13.61^{A}	8.48^{B}	5.41^{C}	1.91^{D}	F=166.5 P=0.21 x 10^{-8}***
% HC yield	5.97^{B}	3.95^{C}	4.13^{C}	8.03^{A}	F=34.5 P=0.40 x 10^{-6}***
g HC/ g DW 10 leaves	0.82^{A}	0.31^{B}	0.23^{BC}	0.15^{C}	F=64.1 P=0.35 x 10^{-7}***

Dry leaf weights declined (Fig. 3) from the more mesic plot (College Station) to more arid plots, reaching a minimum in the driest, harshest plot (Hurricane, UT). % yields HC declined (Fig. 3) in Lubbock and Oslo in the Texas panhandle, but increased, highly significantly, in the harshest site at Hurricane, UT. g HC/ g 10 leaves was highest (Fig. 3) in the College Station plot, then declined, mirroring the leaf biomass.

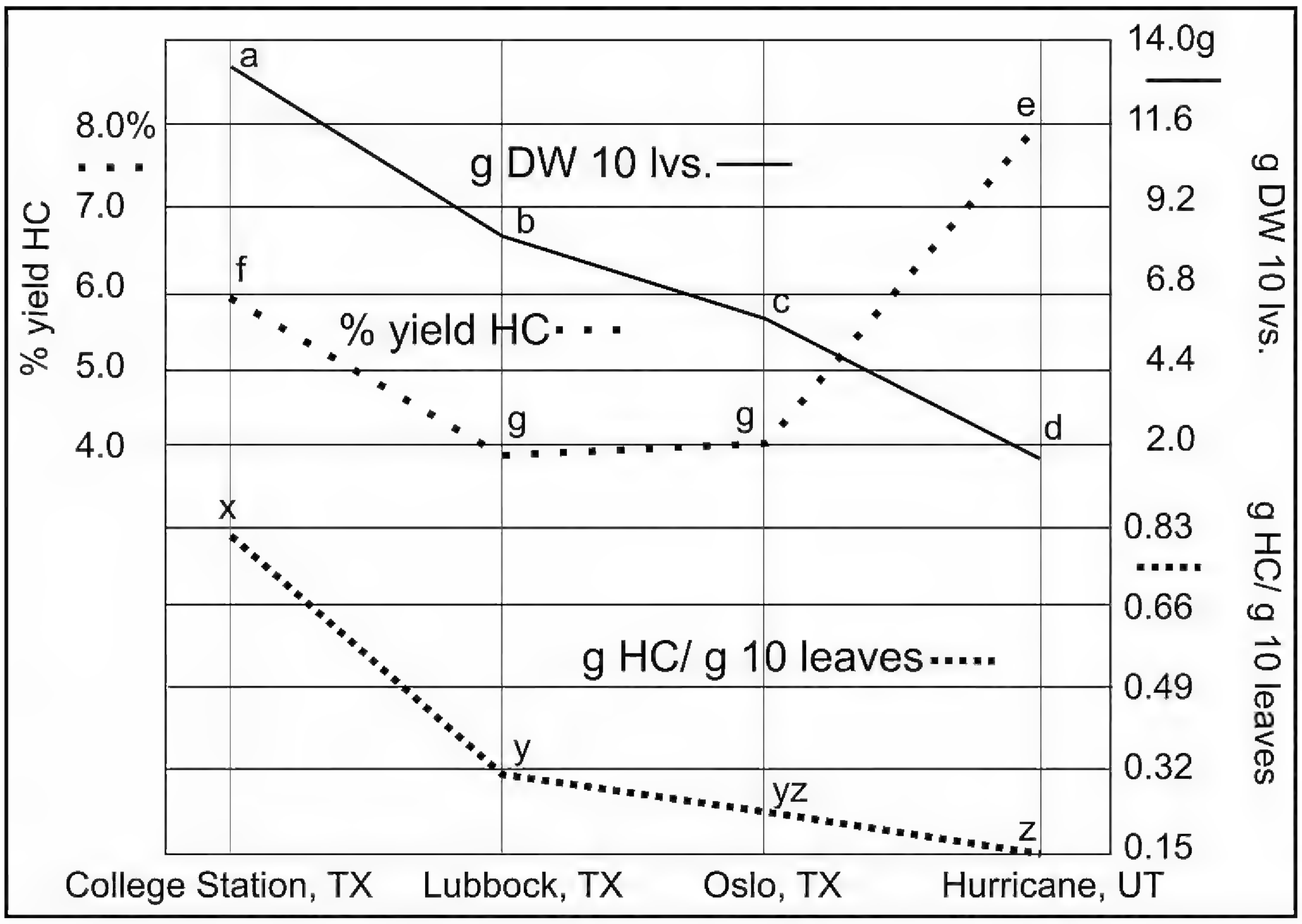

Figure 3. Graphs of leaf biomass (g DW 10 leaves), % yield HC, and g HC/ g DW 10 leaves from accession SA-2269 grown in the summer, 2017 at College Station, TX, Lubbock, TX, Oslo, TX and Hurricane, UT. Any data values that share the same superscript are not significantly differ at P=0.05 by SNK multi-range tests.

This study found very highly significant differences in g DW 10 leaves, % yield HC and g HC/ g DW 10 leaves among the test plots. Leaf biomass and g HC/ g DM 10 leaves declined as expected in the drier plots. However, there was (Fig. 3) a very high, significantly ($P=0.40 \times 10^{-6}$***) larger % HC yield in the arid Hurricane, UT plot.

The plot at Hurricane, UT is in the northeast region of the Mojave Desert at 3277 ft elevation. The seed of SA-2269 were planted on 6 June, 2017. Three plants produced their first flower on 1 Aug, then others flowered until 17 Aug. Plant leaves were harvested when a plant produced its first flower. This is a semi-arid to arid site. 2017 monthly records for Hurricane show (rainfall, avg. high temp., max. high temp.) as: June: 0.0", 100.3°F, 111.0°F; July 1.75", 100.6°F, 113.0°F; Aug 1.44", 97.2°F, 106.0°F; Sep 0.39", 88.0°F, 104.0°F. The plants were watered only as needed for survival.

This study revealed that extreme drought stress can induce the higher synthesis of free HC in cotton accession, SA-2269. However, the trade-off is the lack of biomass production under extreme drought stress. Notice that the g HC/ leaf biomass is very miniscule in the Hurricane, extreme plot. Thus, the actual effect on stress at Hurricane produced lower amount of HC. Nevertheless, study of the bio-induction of free HC in cotton seems a profitable subject for further research.

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Advantages of being a monoecious juniper

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ABSTRACT

This study represents the first report of a monoecious individual of *Juniperus oxycedrus* var. *badia* (a dioecious species), and the reproductive advantages that monoecy appears to confer to that individual in a small population. On the other hand, viable seeds (according to the tetrazolium test) were found in a second isolated female *badia* juniper; that individual should be pollinated by individuals of *J. macrocarpa* because there are no con-specific males in its neighborhood, that is, hybridization may have taken place. Published on-line www.phytologia.org *Phytologia 100(4): 205-207 (Dec 21, 2018)*. ISSN 030319430.

KEY WORDS: *Juniperus oxycedrus* var. *badia*, monoecy, seed viability, sex expression

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Lability of sexual expression has been reported in monoecious and dioecious juniper species (Vasek, 1966; Jordano, 1991; Adams and Thornburg, 2011), as well as in other gymnosperms such as *Taxus* (Allison, 1991). As Adams reported from *J. virginiana*, (and in most all *Juniperus* species) a few monoecious individuals may be found among thousands of dioecious individuals (Adams and Thornburg, 2011). Individuals that change sex may have some reproductive advantages in a population as Freeman et al. (1984) suggested in *Atriplex canescens*, although it has been little studied.

J. oxycedrus var. *badia* is a dioecious, wind-pollinated, long-lived tree up to 15 m, with a pyramidal crown, pendulous terminal branches, triverticillate prickly leaves up to 2 mm broad, and fleshy cones (galbuli) bigger than 1 cm. It produces pollen-bearing cones between November and January, and fleshy cones ripe in October of the second year. In the Iberian Peninsula it shows a continental distribution pattern, with a tendency to colonise the inner regions (Cano et al., 2007). According to Flora Ibérica (Franco, 1986), *Juniperus oxycedrus* var. *badia* is a component of sclerophyllous continental dry woodlands, in an altitude between 200-800 (1000) m. However, a small population has been recently re-discovered in El Rompido (Cartaya) on the coast of Huelva (SW Spain) (Muñoz-Reinoso and Sánchez-Gullón, 2016). Most of the trees are located on a coastal paleocliff (14 trees), four are located on sandy soils at the foot of the cliff sharing the habitat with *J. macrocarpa*, and the last one grows on sandy dunes several kilometers away in the understory of a pine plantation and close to individuals of *J. macrocarpa*. In January 2017, during the flowering season, it was noticed that one of the trees growing on the paleocliff was monoecious (Fig. 1, plant URB013).

The purpose of the present study was to examine the reproductive advantages that monoecy may confer to that individual in this small set of coastal individuals of *J. oxycedrus* var. *badia.*

MATERIALS AND METHODS

In October 2015, 50 female cones were collected from the trees which bore mature cones (10 individuals) near El Rompido, Spain (Fig. 1). Seeds were extracted from each cone, counted and measured with a digital caliper (height, length, width) in order to estimate their volumes by approximation to an ellipsoid (n= 50 seeds). Seed viability was assessed through the cutting method (to determine the percentage of empty seeds) and later, seeds with an embryo were subjected to the tetrazolium test. Juniper height and distance to the closest male juniper were measured.

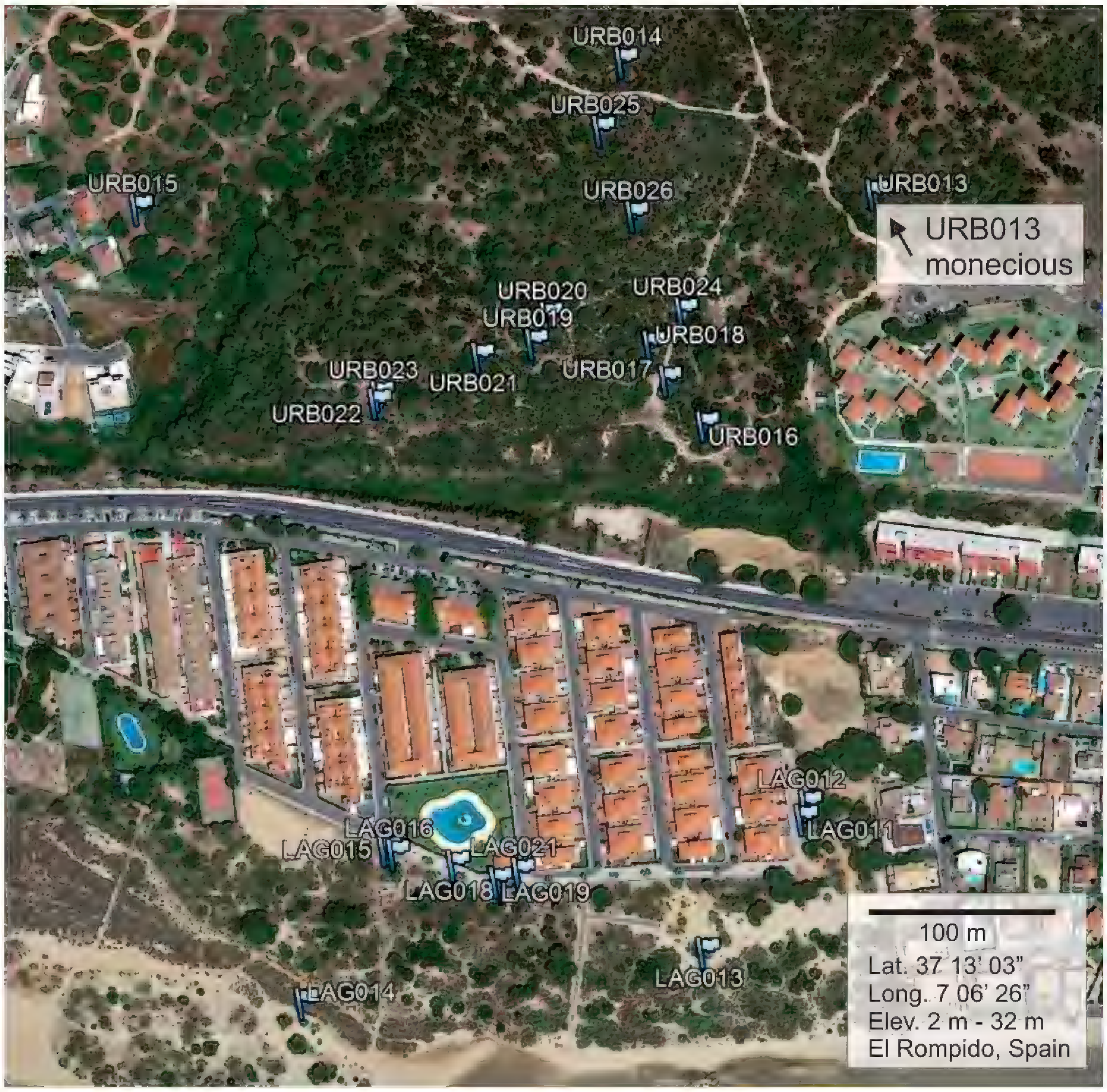

Figure 1. Map showing study area. Note locations of trees sampled. The monecious tree (URB013) is in the upper -right corner. Map modified from Google Earth as per universal agreement for scientific use only and no commercial use.

RESULTS AND DISCUSSION

Mean number of seeds/cone was 1.95±0.15 (mean±s.e.). Except for the monoecious tree (Fig. 1, plant URB013), all junipers growing on the cliff had less than two seeds/cone (mean distance to a con-specific male 72.4±19.8 m), while the three others had among 2.2 and 2.5 seeds/cone. Mean volume of seeds was 72.0±4.5 mm^3, showing the junipers on sandy soils the lowest sizes (42.1-67.3 mm^3) and the junipers on the cliff the highest (69.0-93.7 mm^3). Mean percentage of filled seeds was 50.7±5.7%, while the percentage of aborted embryos was 30.7±2.9%, and the percentage of empty seeds 18.7±4.7%. Finally, the percentage of viable seeds was 26.3±4.1%.

Two things should be highlighted. First, in the monoecious tree (Fig. 1, plant URB013) the proportion of male structures was clearly higher than the proportion of female ones. Monoecy seems to confer to this individual the highest number of seeds/cone (2.78±0.12), the lowest proportion of aborted embryos (16.7%), a very low proportion of empty seeds (6.7%), and the highest seed viability (50.0%) in spite of being to a distance of 120m from a con-specific male.

Secondly, one of the individuals bearing fleshy cones should be pollinated by individuals of *J. macrocarpa* because there are no con-specific males in its neighborhood, that is, hybridization may have taken place, and a high proportion of viable seeds (totally stained) have been produced (33.3%). Hybridization in junipers has been reported by Adams and Wingate (2008) and Adams (2015).

In spite of a lack of germination tests, these preliminary results show advantages that monoecious junipers may have, and the possibility for hybridization between close junipers (Adams, 2000).

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Cistanthe philhershkovitziana (Montiaceae): a remarkable annual species of *Cistanthe* sect. *Cistanthe* from Chile

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ABSTRACT

Cistanthe philhershkovitziana Hershk. (Montiaceae) here is described as the only fundamentally annual species of *Cistanthe* sect. *Cistanthe*, a taxon comprising ca. 12 species in Chile and two in Peru. *Cistanthe philhershkovitziana* is restricted to western Regions IV (Elqui and Limarí Provinces), V (Petorca Province), and Metropolitana (Chacabuco Province) of Chile. All other species are perennial (at least reportedly), although another is facultatively annual. Although uncommon in the southern portion of its range and appearing only in intermittent rainy years in the north, populations of the showy-flowered *C. philhershkovitziana* are conspicuous and distributed along well-travelled highways in populated coastal regions. Its oversight is remarkable and owes to historical inadequacy of the taxonomy of *C.* sect. *Cistanthe*. The origin of the annual life history presumably is derived in *C. philhershkovitziana*, but a caveat is that annuals are ancestral in certain closely related taxa. The species faces no immediate threat in Region IV, but the backdune habitat of the type locality in Region V is severely threatened by human encroachment. Published on-line www.phytologia.org *Phytologia 100(4): 208-xxx (Dec 21, 2018)*. ISSN 030319430.

KEY WORDS: Montiaceae, *Cistanthe*, life history.

As described in Hershkovitz (2018a), *Cistanthe* Spach sect. *Cistanthe* (Montiaceae, Cistantheae) comprises perhaps 12 species in Chile plus another two in Peru. Historically, the taxonomy of the Chilean species has been exceptionally inadequate. The plants never have been monographed, and the most recent revision of the Chilean species is Reiche (1898). Reiche formally recognized only a single species in Chile, *Calandrinia grandiflora* Lindley [= *Cistanthe grandiflora* (Lindley) Schltdl., *nom. cons.* (Veldkamp 2015, Applequist 2016)], which he described as polymorphic. But he also commented on the traits of other described species.

Reiche evidently based his treatment on Philippi's (1893a, b) preliminary studies and analysis of the relatively few herbarium specimens then available in Chile. Reiche's erroneous conclusion has influenced floristic and taxonomic works up to the present day. It also influenced my own thesis research (Hershkovitz 1990a, b, 1991a-c), which also based on studies of herbarium specimens.

But these succulent plants make poor herbarium specimens, because their diagnostic traits are not well-preserved, and the specimens tend to break up and disintegrate with time. Several species are distinguished easily in the field by morphology and locality. Eleven species are diagnosed in Chile by Hershkovitz (2018a), but probably there are at least two more.

One of the species recognized by Hershkovitz (2018a) was unnamed and here is named *Cistanthe philhershkovitziana* Hershk. It is remarkable, because it is a semelparous winter annual, whereas most species of the section are iteroparous perennial herbs and succulent shrubs. Whether any other species are truly annual is not certain. *Cistanthe grandiflora*, might behave facultatively as an annual in drought conditions, but otherwise it is very different from *C. philhershkovitziana*. The possibility that other species may be or behave as annuals is elaborated in the discussion below.

Hershkovitz (2006) erroneously indentified specimens of *C. philhershkovitziana* as *C. grandiflora*, and, concomitantly, specimens of *C. grandiflora* erroneously as *C. discolor* (Schrad.) Spach. Based on my subsequently modified diagnosis, Villagrán et al. (2007) likewise described and illustrated *C. philhershkovitziana* as *C. grandiflora* and, concomitantly, *C. grandiflora* as *C. glauca* (Schrad.) Lilja. In fact, the last diagnosis is correct technically, but *C. grandiflora* subsequently was conserved against *C. glauca* (Veldkamp 2015, Applequist 2016).

My erroneous diagnosis of *C. philhershkovitziana* as *C. grandiflora* was a deduction inadequately researched. Diagnosis of the two sympatric species in Chile's Regions IV and V, *C. glauca* and *C. laxiflora* (Phil.) Peralta & D. I. Ford, was straightforward. The only name remaining for a type from this zone was *C. grandiflora.* I had not considered the possibility that *C. glauca* was taxonomically the same as *C. grandiflora*, and that *C. philhershkovitziana* had been overlooked altogether.

Although uncommon in Region V and appearing only in intermittent rainy years in Region IV, it is reasonably conspicuous. In fact, in rainy years, plants are visible along the shoulder of the Panamerican Highway in Region IV, Chile, even while driving at 120 km/h. With a range extending ca. 300 km along Chile's principal and well-traveled thoroughfares, how could such a distinctive and conspicuous species have been overlooked for more than a century? I attribute the oversight to Reiche's (1898) taxonomy, which fostered the assumption that all plants of *C.* sect. *Cistanthe* are *C. grandiflora.*

METHODS

The present plants were diagnosed on morphological evidence based on the taxonomic discussion of *C.* sect. *Cistanthe* in Hershkovitz (2018a). Plants were collected from the a single locality to replace earlier collections, which were destroyed following study. The locality was georeferenced previously using a GPS. The satellite view and map were generated using Google Maps.

RESULTS

A new species of *Cistanthe* sect. *Cistanthe* here is diagnosed and described.

Cistanthe philhershkovitziana Hershk. sp. nov. TYPE: Chile: Region V (Valparaiso), Petorca Province, La Ligua Municipality, Humedal de Pichicuy, restricted to sands on a ca. 3.5 ha backdune (ca. 70 x 500 m) featuring a rock outcrop projecting 4 m above the surface, 32.347S 71.444W, 8-10 m (Figs. 1, 2), 11 October 2018, *Hershkovitz 18-01* (holotype [F], isotype [P], Figs. 3-5).

Diagnosis. Plants sharing the morphological and molecular genetic characteristics of species of *Cistanthe* sect. *Cistanthe* (sensu Hershkovitz 2006, 2018a) but differing in having the combination of an annual life history and leafless, terete inflorescence culms.

Winter annuals (facultatively short-lived perennials ?), glabrous, caudiciform. **Taproot** fusiform, branched, 7-20 mm broad at the apex, apex truncate. **Caudical rhizome** [= the characteristic vertical subterranean portion of the stem in between the swollen caudex apex and the swollen bases of the rosettes emerging at the soil surface] elongated 1-3 cm, bearing scaly cataphylls, internodes 1-2 mm apart, branch scars sometimes evident at subterranean nodes, usually branched at soil surface, forming 1-5 metameric flowering branches. **Metameric stems** rosettiform at the base, thickened and fleshy, basal internodes not elongated, bearing numerous rosette leaves. **Leaves** simple, succulent, more or less glaucous, narrowly oblanceolate to obovate, up to 10 cm long, leaf base decurrent, broadened and somewhat clasping at the node, apex acute or in northernmost populations rounded. **Inflorescence culms** bulbous and white at the base, with a few unelongated internodes, bearing cataphylls at the nodes, abruptly constricted above the base, stiff, wiry, reddish, terete, internodes elongated, nodes bracteate. **Inflorescence** cymose, ca. 30 cm long, the developing portion somewhat reflexed, rarely branched, leafless, bracteate, basal nodes sterile, 1-bracteate, flowering nodes 8-10, bracts 2, markedly unequal. **Bracts** up to ca. 10 mm long, amplexicaul, ovate, apex acute, becoming papery with age, densely striate with black resinous lines. **Pedicels** elongate, erect in flower, in fruit sharply angled basally, up to 4 cm long in fruit. **Flowers** perfect, autogamous, facultatively cleistogamous. **Sepals** 2, 10-15 mm long, ovate, acute, densely striated with black resinous lines, persistent, enveloping the fruit. **Petals** 5, free, imbricate, flabellate, 2-4 cm long, rose to fuchsia, darker reddish towards the claw, spreading at anthesis, twisting about the ovary after pollination, deciduous. **Stamens** free, ca. 50 in ca. 3 whorls, 12-15 mm long, filaments subulate, pale to white at the base, rose above, margins papillate basally, anthers 1.5 mm long, dorsifixed, purple, pollen yellow. **Pistil** epigynous, syncarpellate, shorter than to as long as the stamens. **Ovary** greenish, ovate, ca. 3 mm long, 3-loculate, placentation axile. **Ovules** ca. 100. **Style** single, 1.5-6 mm long, white. **Stigma** globular, ca. 2x style width, 3-parted, lobed, crests undulating, forming sulcate invaginations, yellowish, papillate, style and stigma withering after anthesis. **Fruit** a valvate capsule, 8-12 mm long, dehiscing basipetally. **Seeds** subglobose, ca. 0.6 mm, micropyle hooked, strophiolate, black, densely covered with short hairs.

Distribution and habitats. *Cistanthe philhershkovitziana* has been observed in Regions IV, V, and Metropolitana of Chile (Fig. 1B). In Region IV, it occurs only during intermittent rainy El Niño years on the coastal plain semidesert steppe of Elqui and Limarí Provinces in between the Panamerican Highway and the coast. A single individual was observed in 1999 along the Panamerican Highway near Los Vilos, Choapa Province. This occurrence may have been anthropogenic, as I never found another individual in this vicinity in subsequent years. In Region V, it has been observed on backdune vegetation at Zapallar and Pichicuy, Petorca Province. In Region Metropolitana, I observed a small population in a sclerophyllous woodland opening near Tiltil, Chacabuco Province, near the border with Petorca Province.

Geographic variability. Dimensions of the plants are developmentally plastic and somewhat different among the three geographical forms: the backdune beach form (B) of the type locality and Zapallar (Villagrán et al. 2007) in Petorca Province, Region V of Chile; the coastal range sclerophyllous woodland clearing (CR) form collected in Chacabuco Province, Region Metropolitana; and the coastal plain semidesert steppe (CP) form of Limarí and Elqui Provinces Region V. Presumably, the CR form also occurs in openings of coast range sclerophyllous woodland of Petorca Province.

B plants have rather more succulent leaves and caudices than CR and CP forms, and the leaves are more glaucous. The root is 1-2 cm broad near the apex, and the caudical rhizome about half as broad. CR and CP caudices and caudical rhizomes are more slender.

Assuming adequate moisture, inflorescences achieve a length of ca. 30 cm, more erect in CR and CP forms, somewhat more ascending to prostrate in B forms. Petal length is usually ca. 2-3 cm in B and probably CR forms (few observations) but 3-4 cm in adequately irrigated CP forms. Petal color is more rose in B forms and more red-magenta in CP forms. Leaves of CP forms are more obovate, usually acute at the apex, but sometimes more rounded.

Phenology. Plants of *C. philhershkovitziana* evidently germinate with the arrival of the winter rainy period characteristic of the Mediterranean climate of north-central Chile. The first rains typically arrive in late May to early June, but the timing and amount of rain varies significantly year-to-year. The CP plants appear only during rainy El Niño years. During The flowering period extends from late September in the northern part of the range to mid-October in the southern.

Additional observations. Chile: Region Metropolitana, Chacabuco Province, Highway F10G (Camino a la Cuesta de la Dormida) 2 km e of Puente Santa Laura, roadside in opening of sclerophyllous vegetation, *Hershkovitz 99-977* [voucher destroyed!]. Region IV, Choapa Province, former two-lane Panamerican Highway 3 km n of Los Vilos at junction with Highway D85, solitary individual in median strip at junction, *Hershkovitz 99-945* [voucher destroyed!], no plants found in this vicinity during several searches in subsequent years, hence possibly anthropogenically dispersed from northern Region IV. Elqui Province, Las Tacas, coastal plain along and east of the Autopista de Elqui (Ruta 5, Panamerican Highway) km 443 at Las Tacas interchange, *Hershkovitz 00-144* [voucher destroyed!], multiple observations at this locality in rainy but not dry years, 2000-2010. Limarí Province, multiple observations in 2010 along the Autopista de Elqui (Ruta 5, Panamerican Highway), ca. km 350-400, and along Routes D540 and D542 towards the coast and Parque Nacional Fray Jorge. Region V, Petorca Province, La Ligua Municipality, Humedal de Pichicuy, *Hershkovitz 03-06* [voucher destroyed!]; Zapallar Municipality [pers. obs., 2004; Villagrán et al. (2007: 181, 216, lámina 71), as *C. grandiflora*], beach area.

DISCUSSION

Diagnosis. *Cistanthe philhershkovitziana* is distinguished easily from other species of *C.* sect. *Cistanthe* by its annual life history and smaller size. Morphologically, it shares no special similarity with any other species in particular. Some other species are similar to *C. philhershkovitziana* in having (mostly) basal leaves and terete culms. These include *C. discolor*, *C. mucronulata* (Meyen) Carolin ex Hershk. (except coastal forms), and *C. aegitalis* (Phil.) Carolin ex Hershk. (Hershkovitz 2018a). But each of these species, besides being perennials, differs from *C. philhershkovitziana* in additional traits. *Cistanthe philhershkovitziana* is readily distinguished from its sympatric relatives, *C. grandiflora* and *C. laxiflora*. The latter can behave facultatively as an annual (Hershkovitz 2018a). I have seen flowering individuals with strict stems on the order of 20-30 cm high, but normally the plants are much-branched and much larger, as much as two meters high and broad and with dinner plate-sized leaves. But, regardless of size, the definitive diagnostic trait is the triquetrous culm (mentioned by Schlectendal 1831: 74), and also the culms are leafy at the base. *Cistanthe laxiflora* is a succulent leafy shrub (Fig. 2B).

Cistanthe cachinalensis (Phil.) Peralta & D. I. Ford was described as an annual with a slender root (Philippi 1860), so it might be considered as a candidate species for plants here referred to *C. philhershkovitziana*. But I believe that *C. cachinalensis* pertains to the weedy and polymorphic *C. grandiflora*, which can behave as an annual under stressful circumstances. *Cistanthe cachinalensis* was described from a collection from 26.5S, elevation 1700 ft (= ca. 500 m). That locality, known then as “Cachinal de la Costa,” corresponds to mountainous fog desert habitat just south of Parque Nacional Pan de Azucar, Region II, Antofagasta Province. Philippi (1860) described it as having leafy stems, and this is evident in the type specimen (holotype: *Philippi s. n.* [SGO (photo!)]). Unfortunately, the most critical diagnostic trait, the triquetrous culm, is not discerned easily in herbarium specimens.

Rosas & Estévez (2010) considered *Calandrinia taltalensis* I. M. Johnst. (holotype: *I. M. Johnston 5284* [G (photo!)]) as a taxonomic synonym of *C. cachinalensis*. Johnston (1929) described the species as appearing to be biennial, but the type specimen also bears numerous stem leaves. The peculiar trait of the *Calandrinia taltalensis* type is the inflorescence form. Compared to other species of *C.* sect. *Cistanthe*, it is more densely cymose, the cymes are erect and not reflexed, and, as Johnston (1929: 37) noted, the pedicels remain short and erect in fruit, rather than becoming elongated and reflexed. In Hershkovitz (2018a), I considered *Calandrinia taltalensis* as a synonym of *Cistanthe cachinalensis*, but I now consider its taxonomic status as unresolved.

A final possible candidate for the annual species is *Calandrinia speciosa* Lehm. (Lehmann 1831: 7, cf. Schlectendal 1831: 74), *non Calandrinia speciosa* Lindley (= *Calandrinia menziesii* (Hook.) Torr. & A. Gray; see Hershkovitz 2018a), *non Calandrinia speciosa* Hook. (Hooker 1835, *nom. inval.*, = *Cistanthe laxiflora* (Phil.) Carolin ex Hershk.)]. The protolog of *Calandrinia speciosa* Lehm. indicates that the culm is terete, but otherwise is not informative sufficiently to diagnose its identity. There appears to be no other information available that would facilitate its diagnosis.

Life history. *Cistanthe* sect *Cistanthe* has been considered to include only perennial species, whereas most species of *C.* sect. *Rosulatae* (Reiche) Hershk. are annuals. Evidently for this reason, Reiche (1898) classified *C. cachinalensis* in his *Rosulatae* grouping rather than *C.* sect. *Cistanthe* (see Johnston 1929: 147). Molecular data (Hershkovitz 2006) demonstrates that, notwithstanding loss of the vouchers, four samples representing all three geographical forms *C. philhershkovitziana* belong to *C.* sect. *Cistanthe*. One sample, *Hershkovitz 03-06* (locality data not included in Hershkovitz 2006) was from the same Humedal de Pichicuy population as *Hershkovitz 18-01*. However, and not insignificantly (see below), existing molecular marker data for *Cistanthe* fail to diagnose species, much less phylogenetic relations between them.

I previously described *C. philhershkovitziana* (as an unnamed species) as annual (Hershkovitz 2018a) based on recollections of field observations and collections, 1998-2010. The collections were destroyed in 2016. On 11 October, 2018, I visited the Pichicuy population specifically to recollect the lost *Hershkovitz 03-06* collection. I discovered that several individuals showed evidence of perenniality, in particular, branch scars along the caudical rhizome (Fig. 5A, B). Up to three such scars were found, although, because of the sensitivity of the site, I did not excavate more than ten individuals. Flowering individuals were scarce in the 2018 drought season relative to rainy years, and most were stunted. However, some of the largest flowering individuals lacked evidence of prior seasonal growth (Fig. 5C, D).

I am inclined to believe that the subterranean branch scars on the caudical rhizome of some individuals also represent current year growth. Previously, I had visited this site only during rainy years and had not noticed thusly scarred individuals. This might have been luck of the draw. In rainy years, plants are abundant, which is not surprising. An individual bearing four inflorescences, each with ten fruits each yielding 100 seeds, would produce 4000 seeds. Finding only annual growth in collected individuals, I was satisfied that the plants were annual. However, 2018 was a drought year with irregularly distributed rainfall. The few passing frontal systems were separated by a few weeks of unseasonably warm weather. The final system passed through on 18 September, 22 days before my collection.

Thus, I suspect that the seeds germinated in response to early winter precipitation, but the moisture was inadequate to sustain reproductive growth. The branches would have died back to the caudical rhizome and abscised, leaving a scar. Plants lacking such scars germinated following a more recent rain, which was sufficient to sustain reproductive development in both these and previously germinated individuals. This die-back morphology evidently is programmed in perennial *Cistanthe* species, in which massive annual above ground growth, including the basal leaf rosette, dies back to the caudex after flowering. *Cistanthe philhershkovitziana* evidently retains this capacity. But whether the caudex can persist for more than one successful reproductive cycle (i.e., whether individuals can be iteroparous) is not clear. This would need to be established experimentally.

My suspicion is that the caudex itself is not persistent, at least not for very long. Caudex persistence would be favored most is a low backdune locality like that in Humedal de Pichicuy, ca. 100 m from the shoreline, where favorable temperature and humidity are maintained year-round, even in drought years. The backdune itself is formed and delimited by the Río Guaquén delta and an estuarial extension (Fig.1A). Thus, the water table remains relatively high. A few massive and evidently very old individuals of *C. laxiflora* occur on the site, and they flower amply even in drought years. Thus, if caudices of *C. philhershkovitziana* indeed persisted several years, one would expect to find larger plants and expect these to be the most conspicuous individuals during a drought year like 2018 (as is the case for *C. laxiflora*!). In fact, the population was rather sparse this year, and most individuals were stunted and formed few flowers. Among the few larger plants was one lacking caudical rhizome branch scars (Fig. 3A, B, Fig. 5C).

I believe that the vestigial perenniality of the B plants is specific to this habitat. I doubt that the CR and CP plants manifest this trait. I no longer possess the specimens to evaluate the caudex of my CR and CP collections. These populations would not have germinated in the drought of 2018. My recollection is that these plants unearthed easily and showed no evidence of perenniality. The CP populations evidently emerge only in El Niño years, when rainfall is sufficient to provoke the "desierto florido" phenomenon in the Atacama Desert further north. One population flowers abundantly in rainy years along the Panamerican Highway at Las Tacas (Region IV, Elqui Province). During one drought season, I thoroughly searched at least two hectares of this locality, digging into the sandy soil, looking for evidence of these plants. I found none.

Among *C.* sect. *Cistanthe*, *C. philhershkovitziana* tentatively is the only species that is fundamentally annual. As noted, *C. grandiflora* may be facultatively annual. But another species meriting investigation is *C. tovarii* Galán de Mera of Tacna Province, Peru (Galán de Mera 1995). This species bears similarities with *C. paniculata* (Ruíz and Pavon) DC, but clearly is distinct in having glabrous seeds and rather reduced flowers. Although described as a perennial, presumably this was based on its thickened root and not based on multiple field observations or experimental evidence. While the illustration manifests a thickened root, it does not appear to be thicker than that of *C. philhershkovitziana*. Given the relatively small size of the plant and the exceptionally reduced flower size, there seems to be a possibility that *C. tovarii* is annual.

Presumably, the annual life history of is derived within *C.* sect. *Cistanthe*, but there are caveats (Hershkovitz 2018b, c): **(1)** as noted above, existing molecular marker data do not diagnose, much less resolve relations among species of the section; **(2)** in *C.* sect. *Rosulatae*, the annual life history is ancestral and perenniality is derived at least twice; **(3)** among other Cistantheae, perenniality is derived in Calyptridinae and (effectively) in *Lenzia*; perenniality presumably is ancestral in *Montiopsis*, but with caveats and, in any case, the high divergence of this genus may render moot is relevance in evaluating ancestral states in other Cistantheae; **(4)** the diverging clades adjacent to Cistantheae are the trans-cordilleran Phemerantheae and Australian *Rumicastrum* Ulbrich; the former include only perennials, the latter are ancestrally annual (Hancock et al. 2018).

Ecology and conservation. *Cistanthe philhershkovitziana* is restricted to open, well-drained sandy soils of backdunes, semidesert coastal plain steppe, and open areas of coastal range woodland. Evidently, it is scarce in the latter vegetation. The most extensive populations spread for many hectares in the coastal plain of southern Elqui and Limarí Provinces. At present, no anthropogenic activity threatens these populations, especially as the plants are robust, autogamous, and highly fecund. But a significant change in precipitation amount, whether more or less, might alter the vegetation and thus indirectly affect the CP range of *C. philhershkovitziana.*

The backdune population at Humedal de Pichicuy is threatened by anthropogenic activity. The small backdune (Figs. 1A, 2.) itself harbors a sparse vegetation of few but almost entirely native species, including those of the genera *Alstroemeria*, *Chorizanthe*, *Leucheria*, *Oenothera*, *Schizanthus*, *Schizopetalon*, and *Tweedia*. Integrity of the backdune substrate is maintained by rhizomes of certain taxa, e.g., *Alstroemeria.* The surrounding denser vegetation, in contrast, is dominated by invasive exotic species. The foredunes are dominated by the exotic *Ambrosia chamissonis* (Less.) Greene. The peculiar conditions of the backdune evidently preserve the native vegetation and resist invasion.

But the backdune vegetation at Pichicuy (and presumably Zapallar) is fragile. The state of the Pichicuy locale is extremely precarious in the face of human encroachment. It is located about 200 m from the escarpment of the Panamerican Highway, 185 km n of Santiago. Prior to 2000, much of this route was two-lane and less traveled. Because of truck traffic and steep grades, travel time to Santiago, especially during vacation periods, was four or more hours. Pichicuy and other smaller beach villages were visited relatively rarely.

With completion of the superhighway in 2001, travel time to Santiago reduced to less than two hours. This, along with dramatic increases in urban population, vehicle ownership, and relative affluence has created demand for permanent and touristic housing and supporting commerce along the coast in Region IV and northern Region V. Today, large housing developments are under construction along the Panamerican Highway immediately opposite the Huaquén-Pichicuy coast, as evident in Fig. 1A. Massive increase in beach access is expected.

During a visit in 2010, I found that the Pichicuy backdune had been severely damaged by motorcycles. Since that time, the Municipality of La Ligua in collaboration with others has taken measures to preserve the Humedal de Pichicuy. Today, the area is posted and guarded. However, massive increase in beach access still threatens the backdune, as it presents an inviting landscape for beach blankets and sand shovels. Hopefully, 24-hour guarding will protect the backdune during the vacation season. Otherwise, unfortunately, conservation may require fencing.

However, more realistically, it must be appreciated that, at a millennial scale, the current Humedal de Pichicuy geography is transitory. Natural processes are bound to modify the coastline and the Río Guaquén delta and estuary. A major tidal wave in this seismically active zone is not inconceivable, and the entire site could be obliterated in seconds. Whether anthropogenic or not, climate change and/or flow reduction or contamination of the Río Guaquén and/or a rising in sea level will eliminate the existing estuarial system and the backdune vegetation.

Whether or not anthropogenic effects present localized and direct threats to the dune/estuary ecosystem, anthropogenic effects at the regional scale likely do. In particular, regional alteration of the coastline undermines the capacity of the natural coastline to mitigate the impact of long-term geographic and climatic changes. Over time, dunes and estuaries and their biota naturally “migrate” and evolve in response to changing conditions. However, highly urbanized coastlines cannot accommodate such natural transformations. From Fig. 1A, it should be evident that the dune/estuary system at Pichicuy, only some tens of meters broad, is constrained on all sides and has no margin for natural movement.

Etymology. *Cistanthe philhershkovitziana* is named as a tribute to my late father, noted zoologist Philip Hershkovitz (1909-1997). This might seem peculiar, given that P. Hershkovitz was not a botanist and never visited Chile. Despite the latter, one of only 17 Chilean endemic mammal species, *Akodon hershkovitzi* Patterson, Gallardo & Freas, 1984, is named in his honor. The discovery and description of *Cistanthe philhershkovitziana* owes no less to his legacy.

In 1998, I was recruited to finance and develop a plant molecular systematics research program in Chile, and I moved there in January, 2000. Despite the considerable personal economic cost and professional inconvenience, Philip Hershkovitz had instilled in me philanthropic convictions, and I believed that “giving back” to South America was a noble and philanthropic way to both extend his legacy and expend his largesse. I was a naïve liberal progressive. Long story short, by 2016, I found myself homeless on the streets of Santiago.

Among the very few possessions I have retained is a gift from P. Hershkovitz, a personalized copy of his 1977 Living New World Monkeys tome (Hershkovitz 1977), a fraction of his lifetime work (Langguth 1997, Mittermeier 1997, Patterson 1997). As an adolescent, I also labored countless hours on this effort. Now, with a critical eye, I appreciate its unequaled scholarly qualities. But no living beneficiary of the work of P. Hershkovitz can appreciate more than I the history of poverty, persecution, sacrifice, valor, and tragedy on which it was wrought. For example, P. Hershkovitz (1977) cites specimens he collected in Ecuador, 1933-1937, where he moved because the Great Depression in the US left him unemployed, broke, and unable to pay his undergraduate tuition. He travelled alone in the jungle, hunting his own food, and constructing his own lodgings. So sown was the seed of his monumental achievement.

But even considering the comparatively comfortable labor conditions of the Field Museum in the 1950s-1960s, it may be difficult for modern researchers to conceptualize how P. Hershkovitz' bibliography could have been generated without the benefit of modern digital—in some cases even electric—gadgetry. As was the norm in taxonomy in those days, P. Hershkovitz' work, including much of his 1977 tome, was drafted and corrected entirely in pencil and, before 1970, typed on a manual typewriter with two carbon copies. All (analog) photography, illustration, plate arrangement, and labeling (one of my principal tasks) were done manually. Likewise, modern researchers may not be able to conceptualize how such a bibliography was generated without the cumulative career assistance of the current norm of scores to hundreds of coauthors.

Reflecting on this, I appreciated that my current conditions, however difficult, must be considered comparatively privileged and posh compared to the Ecuadorian jungle in the 1930s. My father's literary incarnation thus motivated my renewed efforts to write manuscripts and, in particular, describe this particular species, which I recollected on the eve of P. Hershkovitz' 109th birthday. Two of P. Hershkovitz' works were all I had to weight the makeshift plant press. It would be a travesty for me to name the species in any other manner. Appropriately, the holotype has been deposited in the herbarium of the Field Museum in Chicago, where P. Hershkovitz labored, 1947-1997.

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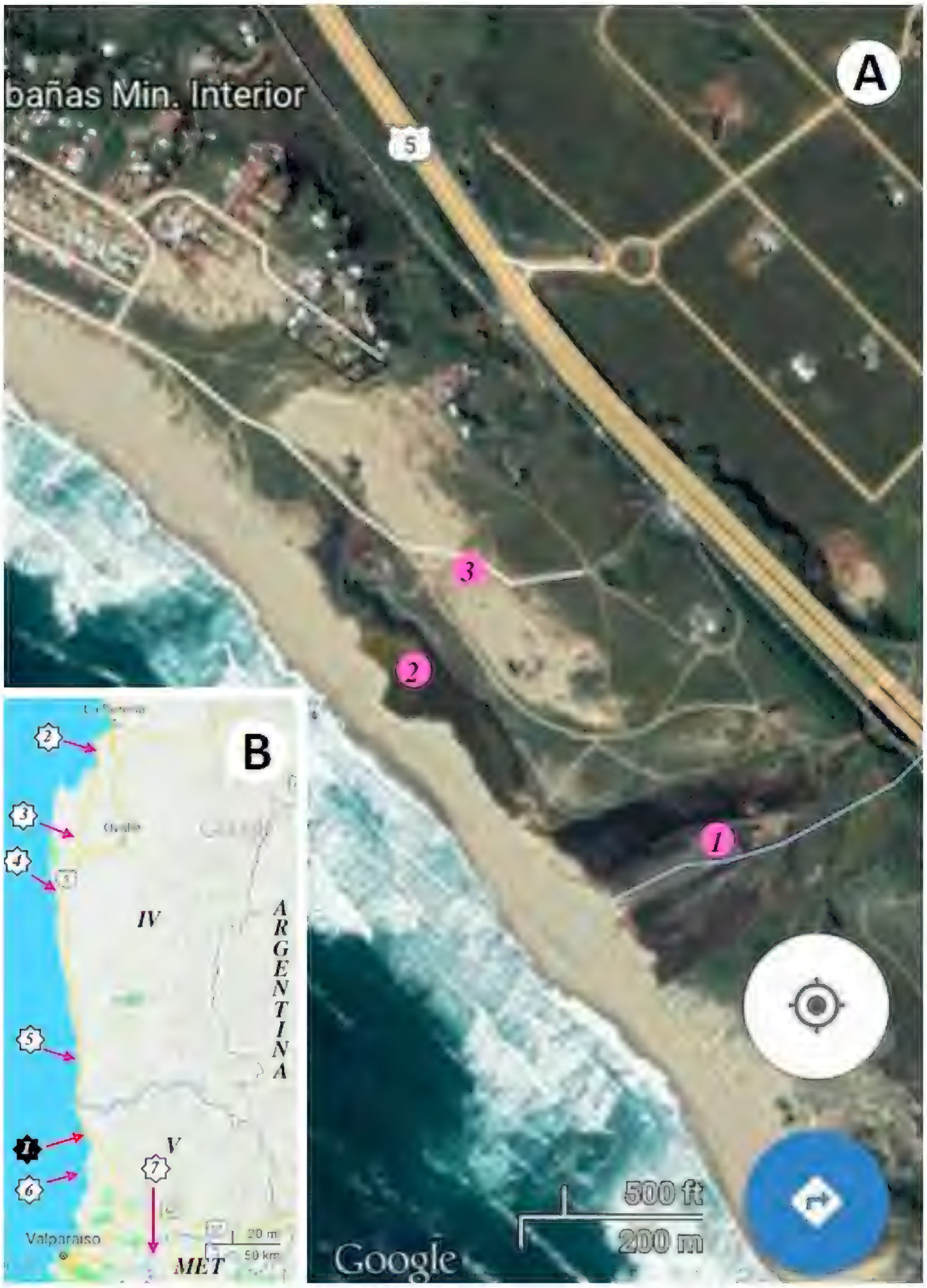

Fig. 1. Locality data for *Cistanthe philhershkovitziana*. **A.** Satellite view of the type locality, a portion of Humedal de Pichicuy. A portion of the older section of the community of Pichicuy is at the upper left. A portion of a new housing development under construction is visible in the upper right, separated by the Panamerican Highway (Ruta 5). ***1.*** The mouth and delta of the Río Guaquén. This is the border between the Municipalities of La Ligua and Papudo (light blue line). ***2.*** Lateral estuary of the Río Guaquén. ***3.*** Backdune. Plants of *Cistanthe philhershkovitziana* are restricted to this oblong areas (lighter in color), especially the southeastern portion near the rock outcrop (darker spot). **B.** Map of north-central Chile showing collection/observation localities of *Cistanthe philhershkovitziana* (see text) in Regions IV, V, and Metropolitana (MET). ***1.*** Type collection locality, Pichicuy. ***2-7.*** Previously collected localities (vouchers destroyed). ***2.*** L as Tacas. ***3.*** Between the Panamerican Highway and Parque Nacional Fray Jorge. ***4.*** Along the shoulder of the Autopista de Elqui (Ruta 5, Panamerican Highway), ca. km 350. ***5.*** North of Los Vilos along the prior Panamerican Highway. ***6.*** Beach in the vicinity of Zapallar. ***7.*** Chacabuco Province.

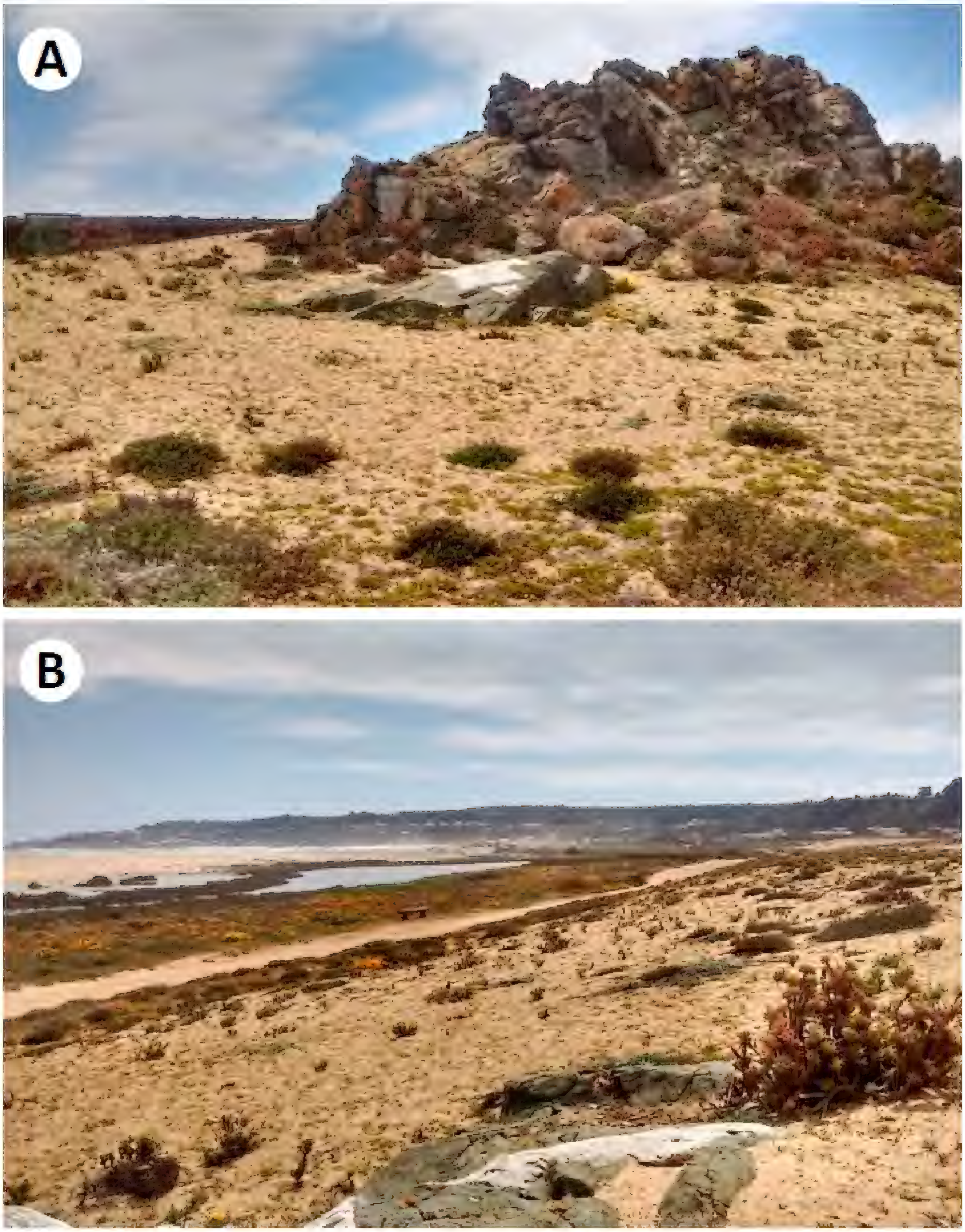

Fig. 2. Humedal de Pichicuy backdune habitat of *Cistanthe philhershkovitziana*. **A.** View eastward towards rock outcrop. **B.** View westward away from rock outcrop towards estuary, beach, and the village of Pichicuy. The succulent shrub in the foreground is a modest-sized individual *Cistanthe laxiflora*.

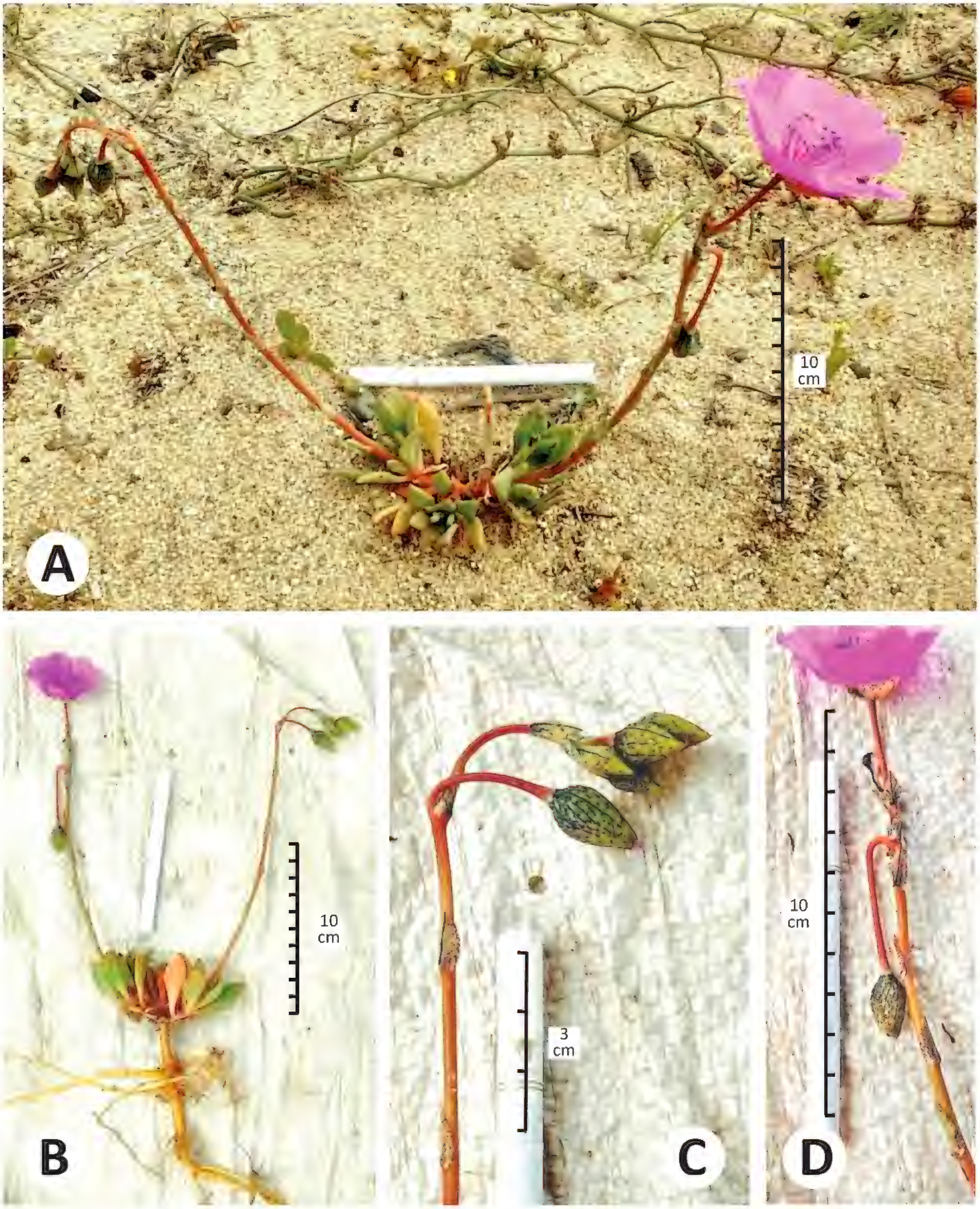

Fig. 3. *Cistanthe philhershkovitziana. Hershkovitz 01-18.* **A.** Naturally occurring plant. B. Whole plant excavated. **C.** Developing portion of inflorescence. **D.** Reflected pedicel after anthesis.

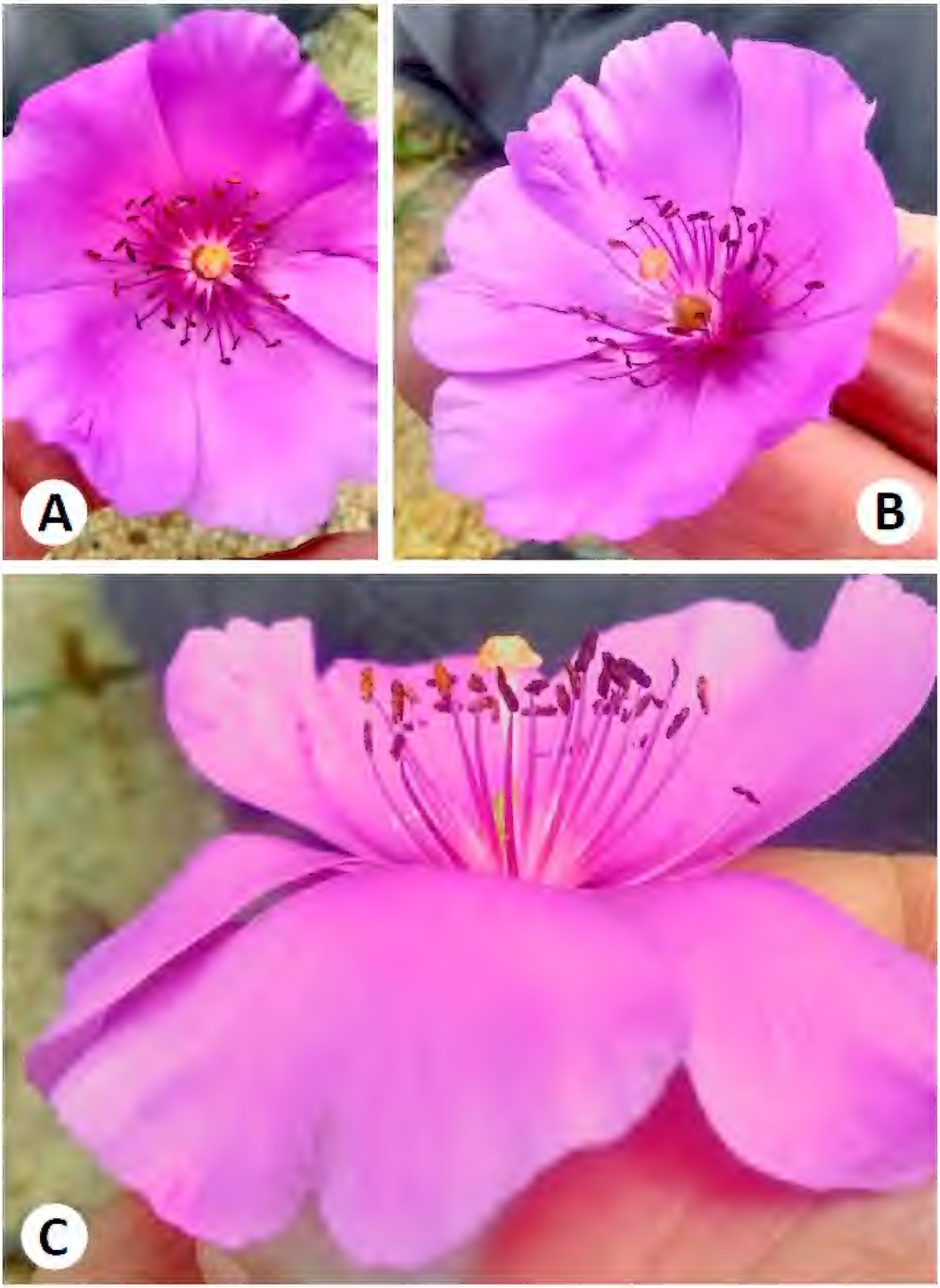

Fig. 4. Flower of *Cistanthe philhershkovitziana*. *Hershkovitz 01-18*. **A.** Radial view. **B.** Oblique view. **C.** Lateral view.

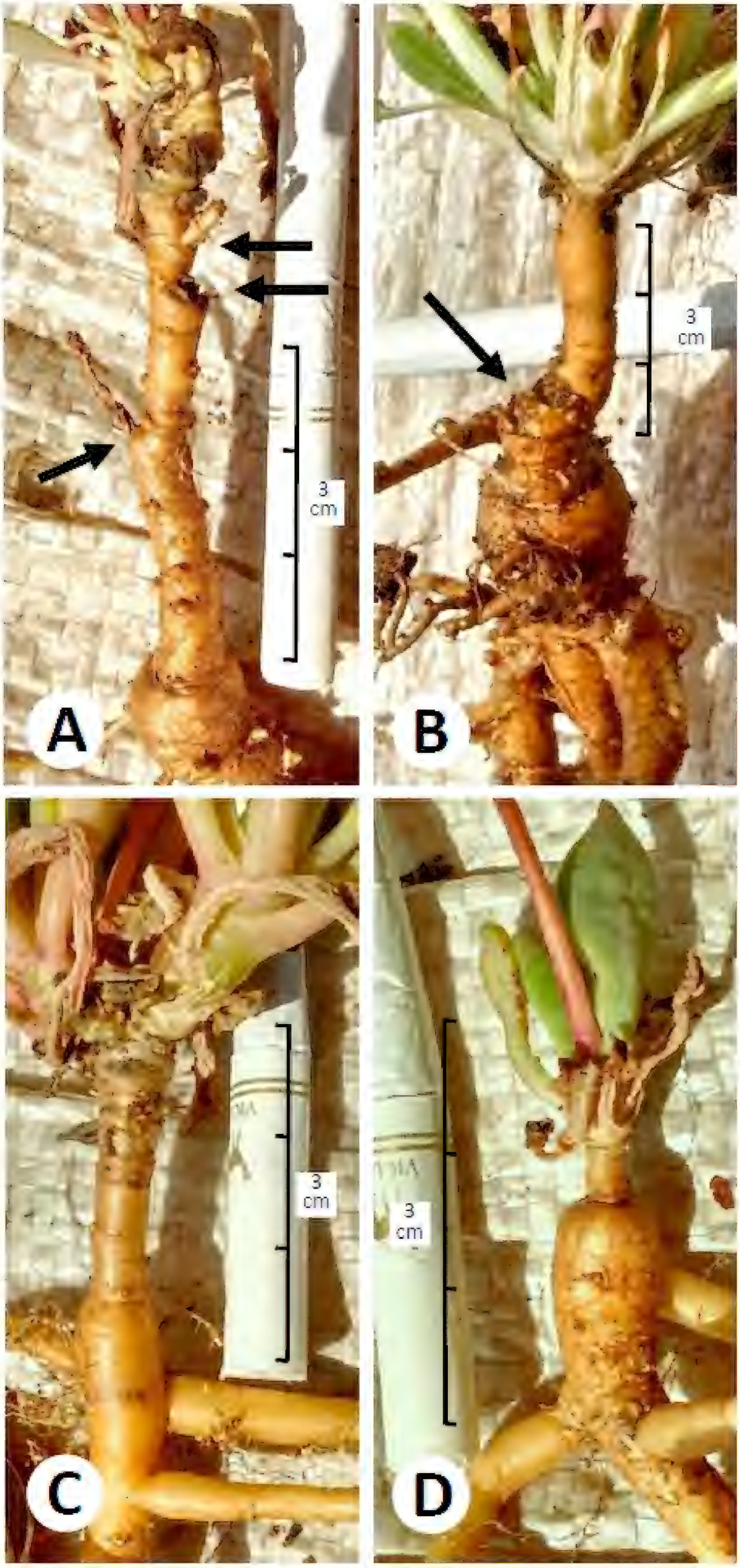

Fig. 5. Caudices of *Cistanthe philhershkovitziana*. **A.** Caudex with three branch scars (arrows) along the caudical rhizome, below the extant leafy rosette. **B.** Caudical rhizome with a subterranean central branch scar (arrow) and lateral subterranean branch supporting the extant leafy rosette. **C.** Close-up of the caudex apex and caudical rhizome of the flowering specimen in Figure 1, lacking caudical rhizome branch scars. **D.** Additional flowering specimen lacking caudical rhizome branch scars.

The Whitebeam problem, and a solution

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ABSTRACT

The position of ***Sorbus*** *sensu lata* within the **Malinae** (**Rosaceae**) and in the context of the historical treatment of five European Sorboid genera is reviewed, with an appraisal of published molecular studies. The treatment proposed here is to accept ***Sorbus*** L., ***Cormus*** Spach, ***Aria*** (Persoon) Host, ***Chamaemespilus*** Medikus, ***Torminalis*** Medikus, ***Micromeles*** Decaisne and ***Pleiosorbus*** Zhou & Wu and the five genera of hybrid origin proposed by Sennikov & Kurtto (***Hedlundia***, ***Borkhausenia***, ***Karpatiosorbus***, ***Majovskya*** and ***Normeyera***) and to describe ***Griffitharia*** Rushforth, ***Alniaria*** Rushforth, ***Thomsonaria*** Rushforth, ***Dunnaria*** Rushforth and ***Wilsonaria*** Rushforth as new genera for the Asiatic whitebeams. The genera in the Maloid group are reviewed and ***Malus*** Miller, ***Eriolobus*** (A.P. de Candolle) M. Roemer, ***Chloromeles*** (Decaisne) Decaisne, ***Macromeles*** Koidzumi, ***Docynia*** Decaisne and ***Sinomalus*** Koidzumi accepted and ***Prameles*** Rushforth described. Various new combinations are made. A key to the 45 genera recognised in the **Malinae** is presented. Published on-line www.phytologia.org *Phytologia 100(4): 222-247 (Dec 21, 2018).* ISSN 030319430.

KEY WORDS: Sorbus, Malinae, Rosaceae, taxonomy.

In a seminal paper looking at morphological characters Robertson, Phipps, Rohrer & Smith (1991) (hereafter Robertson *et al*) reinstated the treatment coherently expressed by Roemer (1847) that the European "Sorboids" comprise five separate genera. These genera are *Sorbus* L. [1753], *Torminalis* Medikus [1789], *Chamaemespilus* Medikus [1789], *Aria* (Persoon) Host [1831] and *Cormus* Spach [1834]. These were all sunk, along with *Aronia* Medikus [1789], *Eriolobus* (A. P. de Candolle) Roemer [1847] and *Micromeles* Decaisne [1874], by Wenzig (1883) into his concept of *Sorbus*. Rehder (1915, 266—279) espoused Wenzig's concept of *Sorbus*, which by the early 1920s had superseded the wider use of *Pyrus* L. [1753] for the "Sorboid" genera. For much of the late 19th and early 20th centuries *Pyrus* was frequently used for both rowans (*Sorbus sensu stricto*) and whitebeams (*Sorbus sensu lata*), and for several other genera, including for some authors *Malus* Miller [1754], although Rehder kept *Malus* separate from *Sorbus* and *Pyrus*. However, Robertson *et al* pointed out the "uncomfortable fact" that the whitebeams in *Sorbus sensu lata* are closer to the apples (*Malus*) than to the rowans.

Two problems with Robertson *et al* – perhaps described as a shortcoming and as a flaw – were that a key to the genera was not provided and, more significant in the context of the Whitebeam problem, the many European microspecies derived from hybridisation between *Aria edulis* (Willd.) M. Roemer and *Chamaemespilus alpina* (Miller) Robertson & Phipps, *Sorbus aucuparia* L. and/or *Torminalis clusii* (M. Roemer) Robertson & Phipps—which due to their apomictic breeding system form a significant part of the native woody flora in parts of Europe and Asia east to Iran—were left in limbo without a usable generic name.

Since Robertson *et al,* molecular investigations have become available. In the Rosaceae there are four major papers covering the group, whether as Maloideae (traditional treatment as a subfamily of Rosaceae) or as Pyrinae, Pyreae or Malinae (as a subtribe in the Amygdalineae). These are Campbell *et al* (2007), Potter *et al* (2007), Li *et al* (2012)[1] and Lo & Donoghue (2012). These papers all suffer from a very limited sampling of Sorboid genera and taxa. In Campbell *et al*, the Sorboids are: *Aria alnifolia* (Siebold & Zuccarini) Decaisne, *Chamaemespilus alpina*, *Cormus domestica* (L.) Spach, *Sorbus aucuparia* and *Torminalis clusii*. In other genera one to three species were sampled. Note the type species of *Aria* (*Aria edulis*) was not included in this analysis. Potter *et al* used a similar small set of taxa, not surprising when the five authors of Campbell *et al* are included in the eleven authors of Potter *et al*.

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[1] Hereinafter Campbell *et al*, Potter *et al*, and Li *et al*, similarly for Aldosoro *et al*, Qian *et al* and Guo *et al.*

In Campbell *et al*, using cpDNA [fig 1] *Aria* (*alnifolia*)[2] is well removed from *Cormus* Spach and *Sorbus* L. with *Pyrus* L. sister to *Sorbus* with *Cormus* basal to these two genera, and with *Chamaemespilus* Medikus and *Torminalis* Medikus close together in an unresolved separate clade including *Malus*, *Docyniopsis* (C. K. Schneid.) Koidzumi[3] and five other genera (out of the 28 Malinae genera included in the sampling). Fig 2, using data from the granule-bound starch synthase gene GBSSI-1A *Aria* (*alnifolia*) and *Chamaemespilus* are sister with *Torminalis* basal and with this clade sister to *Cormus* and *Sorbus* in a clade which is sister to *Pyracantha* M. Roemer [1847]. With GBSSI-1B, fig. 3, *Cormus* and *Sorbus* are in a clade which is sister to a poorly resolved clade which includes all the other 14 genera in the figure. In the poorly resolved clade, *Aria* (*alnifolia*) and *Chamaemespilus* are sister to each other but in an ill-defined group including *Malus* and *Docyniopsis* and two other genera. Note in this figure *Torminalis* is not present as presumably it does not have this gene. Using GBSSI-2A, fig. 4, all five Sorboid genera are in a poorly resolved clade, with *Aria* (*alnifolia*) close to *Chamaemespilus* at the top of the clade, *Cormus* in the middle and *Torminalis* sister to *Pyrus* with *Sorbus* near the bottom of the clade. In Fig 5, using GBSSI-2B, *Aria* (*alnifolia*) and *Chamaemespilus* are sister to a group including *Cormus*, *Docyniopsis*, *Eriolobus* and *Malus* but with *Sorbus* basal to 16 of the 22 genera in this cladogram (which again excludes *Torminalis*). Combining nrITS with GBSSI-2B in Fig. 6 has *Sorbus* basal to 22 other genera, with *Aria* (*alnifolia*) close to *Chamaemespilus* and sister to a clade containing *Cormus*, *Docyniopsis*, *Eriolobus*, *Malus* and *Chaenomeles* Lindley and *Torminalis* sister to a clade which includes *Pyrus* and *Cotoneaster* Medikus along with unresolved *Osteomeles* Lindley and *Photinia* Lindley (actually *Pourthiaea villosa* (DC) Decaisne). Fig. 7 which includes a dozen non-molecular characters gives a broadly similar arrangement to figure 6.

Figures 1 to 4 in Potter *et al* present a generally similar picture, with *Sorbus* basal to the group and *Aria* (*alnifolia*), *Chamaemespilus*, *Cormus* and *Torminalis* more closely associated.

Lo & Donoghue sampled a much larger number of species and individuals. From Appendix A, which was not part of the published paper but accessible in the online version, in *Sorbus s.l.* their sample included 3 individuals of *Aria edulis*[4] as well as 46 of the mainly hybrid[5] apomictic tetraploid European and West Asian taxa (all as subgenus *Aria*), two of *Chamaemespilus alpina*, two of *Cormus domestica*, 22 assorted individuals of Sino-Himalayan origin as "*Micromeles*" [including *alnifolia*], 124 individuals of *Sorbus s.s.* – mainly Asiatic microspecies but including three samples each of *aucuparia*, *americana* Marshall and *commixta* Hedlund, and two individuals of *Torminalis clusii*. The species listed as belonging to the "*Micromeles*" group are *alnifolia*, *caloneura* (Stapf) Rehder, *epidendron* Handel-Mazzettii, *folgneri* (C. K. Schneider) Rehder, *hedlundii* C. K. Schneider, *hemsleyi* (C. K. Schneider) Rehder, *japonica* (Decaisne)

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[2] I am putting the specific name of the specimen examined by Campbell *et al* after *Aria* as the analysis did not include the type species of *Aria* but a species which I consider is better placed in a separate genus

[3] *Macromeles tschonoskii* Koidzumi

[4] As *Sorbus aria* Crantz

[5] Using the segregate genera in Sennikov & Kurtto [2017] for the apomictic European and West Asian microspecies the species listed in Appendix A belong to tetraploid *Aria* and *Borkhausenia*, *Hedlundia* and *Karpatiosorbus*, as below, but some of their West Asia taxa were not allocated to genera by Sennikov & Kurtto as their treatment is only for the European taxa

Rehder, *lanata* (D. Don) Schauer, '*melanocarpa*'[6], *pallescens* Rehder, *thibetica*[7], *tsinglingensis* C. L. Tang, *verticillata* Merrill, *vestita* (Wallich ex G. Don) Loddiges and *yuana* Spongberg.

Lo & Donaghue's combined chloroplast tree (left hand side of fig. 1) resolved three major clades, viz. **A** *Amelanchier—Crataegus*[8], **B** *Aria—Malus* and **C** *Cotoneaster—Pyrus—Sorbus*, with *Pyracantha* unresolved with **A** and **B-C**. In this presentation *Cormus* sits as a sister to *Sorbus* and "*Micromeles*". *Torminalis* and *Chamaemespilus* are not shown.

The right hand side of the figure shows the result from the nuclear ribosomal ITS DNA sequencing. Including *Pyracantha* all the genera shown are initially unresolved. However, apart from this the *Amelanchier—Crataegus* clade is identical to that from the chloroplast DNA sequencing. The main differences are that *Malus—Aria—Cotoneaster* form a clade with "*Micromeles*" siting between *Malus* and *Aria*, with *Pyrus*, *Pourthiaea—Aronia*, *Cormus* and *Sorbus* as unresolved. Again, *Chamaemespilus* and *Torminalis* are not shown.

The position of "*Micromeles*" occurring as sister to *Sorbus* in the chloroplast DNA sequencing and as sister to *Aria* and close to *Malus* in the nuclear ribosomal ITS DNA sequencing is used to suggest a hybrid origin for this group. However, the disparate cluster of distantly related taxa from eastern Asia, and possibly including North American *Aronia melanocarpa* (Michx.) Britton, may also provide an explanation for this.

Figure 2 suggests a chronology based on combined chloroplast and ITS data. This diagram excludes the "*Micromeles*" taxa. It lists *Torminalis clusii* as basal to a group of *Aria* species which includes *Chamaemespilus alpina*. However, it is disappointing that all the *Aria* taxa listed are part of the hybrid or apomictic Sorboids and include tetraploid *Aria* and apomictic hybrids which include *Aria edulis*, *Sorbus aucuparia* and/or *Torminalis clusii* in their genetics (but not *Chamaemespilus*) but that data from the three collections of *Aria edulis* is not included. In the clade including *Sorbus*, *Pyrus* and *Cotoneaster*, *Cormus* is basal to *Sorbus* and together they are sister to *Pyrus*.

Figure 4 adds nine non-molecular characters to the combined chloroplast and nuclear ITS combined data. This has *Torminalis* as basal to a sister group of *Aria* and *Chamaemespilus* and well removed from the clade which has *Pyrus* basal to a sister pair of *Cormus* and *Sorbus*.

It is pertinent to note, as a word of caution, how in figure 2 one of the two specimens analysed as *tschonoskii* comes out, as *Docyniopsis tschonoskii* with the two species of *Docynia* but another one (as *Malus tschonoskii*) is centred in the *Malus* cluster. As *tschonoskii* is one species, whether placed in *Malus* as Maximowicz[9] named it or in *Macromeles* and *Docyniopsis* where Koidzumi put it, it is likely that one – I suspect the *Malus tschonoskii* sample – has been mis-identified.

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[6] This is recorded as from Ness Botanic Garden and presumed to be *megalocarpa* unless it was *Aronia melanocarpa* from elsewhere

[7] Two collections are listed as *thibetica*. One is Clark & Sinclair 1546 from Edinburgh; this collection is from Bhutan and is *karchungii*. The second is cited as from Sichuan Expedition [SICH] 1464 but with the locality as 'China; Dali; Huadianba'. However, SICH 1464 is not *thibetica* but appears to be *ambrozyana* from China, Sichuan, Xichang xian on the northern flank of Luoji shan. The whitebeam from Huadianba at the northern end of the Cang shan is *guanii*, from CLD 1501, thus neither is *S. thibetica* (Cardot) Handel-Mazzetti.

[8] Genera in the clades are: *Amelanchier* Medikus, *Crataegus L.*, *Pyracantha* M. Roemer, *Pourthiaea* Decaisne

[9] *Malus tschonoskii* Maximowicz, *Docyniopsis tschonoskii* (Maxim.) Koidzumi = *Macromeles tschonoskii* (Maxim.) Koidzumi

The omission of *Aria edulis* from their figure 2 cladogram raises the prospect that their data from this—the type species of *Aria*—was excluded from the other figures and thus questions the usefulness of the references to *Aria* in the other cladograms.

Li *et al* Table 1, listing the species, has three as *Aria* (*coronata* (Cardot) Ohashi & Iketani, *hemsleyi* (C. K. Schneid.) Ohashi & Iketani and *yuana* (Spongberg) Ohashi & Iketani) but not the type species, two specimens of *Chamaemespilus alpina*, two of *Cormus domestica* and three samples of *Torminalis clusii*. Six samples are listed as *Micromeles*. These are three as *alnifolia*, and one each as *caloneura* Stapf, *thomsonii* and *tsinlingensis*, with the above caveat about the placing of *alnifolia*. The sample listed as *thomsonii* is of interest, as it is recorded from Jiangxi and likely to have been mis-identified. Twenty one samples are listed as *Sorbus*, including two of *aucuparia*.

Li *et al* produce two cladograms. In Fig. 1 on nrDNA ITS sequences *Sorbus*, *Torminalis*, *Cormus* and a cluster of taxa listed as variously *Aria* or *Micromeles* (but not including the type species of either genus) all come out in different clades. Fig. 2 gives a similar arrangement. In the *Sorbus* clade, the presence of the *Sorbus aronioides* Rehder sample suggests this was mis-identified.

Combining these four investigations, they provide support for separating the genera *Chamaemespilus*, *Cormus* and *Torminalis* from *Sorbus*. However, it is not possible to draw any conclusions on the placement of *Aria*, as none of them present any data on *Aria edulis* even though three samples were included in the Lo & Donaghue (2012) sampling. The presented results show *Sorbus* and usually *Cormus* separate from *Chamaemespilus* and *Torminalis*, with the former two genera closer to *Pyrus* and the latter pair closer to *Malus*. Thus even without results for *Aria edulis* the maintenance of *Sorbus sensu lata* is not coherent.

When presented with conflicting factual data there are two opposite ways in which to rationalise it. Rehder (1915, p276) gave his reasons for not accepting *Micromeles* as a genus with "Decaisne, who founded the genus *Micromeles*, distinguishes it from his *Aria* chiefly by the smaller flowers, the epigynous disk and the deciduous calyx. The difference in the size of the flowers is not very marked and has hardly any significance as a generic character. In typical *Aria* as represented by *Sorbus Aria* which Decaisne figures, the disk is certainly perigynous, but in such species as *S. cuspidata* Hedlund[10] (*Aria lanata* Decaisne), *S. japonica* Hedlund and *S. alnifolia* K. Koch enumerated by Decaisne under *Aria*, the disk is distinctly epigynous as in the species referred by him to *Micromeles*. The deciduous calyx is not a distinguishing generic character with Decaisne, as he includes *S. japonica* and *S. alnifolia*, which both have a deciduous calyx, in *Aria*."

Rehder took the view that as *Micromeles* and several other whitebeam species known at the time had epigynous disks this provides justification for uniting them with *Aria edulis* and its perigynous disk. However, Decaisne's text (1874) describes his concept of *Aria* as having a perigynous disk, which Rehder agrees is shown in the illustration of *Aria edulis* [as *A. nivea* Host]. A likely conclusion is that Decaisne did not have good flowering material of the other taxa which he included in his *Aria* concept, which Rehder 40 years later was able to correct. Rehder also overlooked Decaisne's reference to the small fruit in his characters separating *Micromeles* from *Aria*.

When faced with new information one option is to retrench, as Rehder did with his treatment of *Sorbus sensu lata*. However, the other option is to see how existing views need amending and thus what changes might be needed to accommodate the new data.

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[10] nomen illegitimate (or superfluous?) as *Pyrus vestita* Wallich ex G Don predates Spach's *Crataegus cuspidata* which is based on the same Wallich specimens

Recently the retrenchment approach to the conflicting molecular data currently available has been taken as justification by Fay & Christenhusz (2018) for treating almost all the group as species of *Pyrus*. This has required the making of 843 new combinations and has obliterated the last two centuries of research. It also totally fails to provide any clarity on the relationship of the thousand or so species of *Pyrus* thereby created and in my opinion is a short sighted and retrograde step not supported by any available published data. Thus whilst at one level it has a degree of logic[11] it fails the primary purpose of taxonomy which is to assist our understanding of plants (or animals, fungi, etc.) and their relationships.

Fay & Christenhusz cite as justification for their approach the treatment of European hybrogenous taxa by Sennikov & Kurtto (2017) where rather than create hybrid genera to contain the numerous microspecies they treat them by creating normal genera of hybrid origin, reflecting the fact that as fertile apomictic species they behave as such rather than as one-off hybrids. Their units are *Hedlundia* Sennikov & Kurrto derived from hybrids between *Aria x Sorbus*, *Borkhausenia* Sennikov & Kurtto for the three-way hybrid derived from *Aria x Sorbus x Torminalis*, *Karpatiosorbus* Sennikov & Kurtto for *Aria x Torminalis*, *Majovskya* Sennikov & Kurtto for *Aria x Chamaemespilus* and *Normeyera* Sennikov & Kurtto for *Aria x Chamaemespilus x Sorbus*, with *Aria* retained for the tetraploid apomictic forms believed to be derived from a doubling-up of the *Aria edulis* genome, or parts thereof. Sennikov & Kurtto's approach has been criticized for not following the standard treatment of creating hybrid genera (incorporating some letters from each of the postulated parental genus names). Personally I find their approach more credible than the hybrid genera approach as their genera are not one-off hybrids but dynamic naturally occurring microspecies.

However, regardless of whether Sennikov & Kurtto's genera are accepted with their names, they neatly and clearly define the groups of European apomictic taxa at the genus level. Thus they overcome the flaw in Robertson *et al*'s treatment and provide an approach for treating the European apomictic hybrid species. They do, however, leave a small number of West Asian apomictic taxa of similar hybrid parentage (some possibly with *Torminalis orientalis* (Schonbeck-Temesy) Robertson & Phipps as one component rather than *T. clusii*) needing new combinations into the hybrid genera.

With the status of the European and West Asian apomictic hybrid Sorboids settled by following Sennikov & Kurtto's approach [whether using their names or their concepts of the groups] the last remaining constraint to following Robertson *et al*'s revival of Roemer's five European Sorboid genera is the provision of appropriate genera for the non-European Sorboids. These are the various whitebeams found in Asia east of Iran and extending south into Sumatra. Whilst *Sorbus* is recorded in North America, the species all belong to *Sorbus sensu stricta* (McAllister 2005).

Aldosoro *et al* (2004) provided a monograph of part of *Sorbus s.l.*, treating *Sorbus* as having four subgenera, viz. *Sorbus*, *Cormus*, *Aria* (including *Chamaemespilus*) and *Torminalis* (as *Torminaria* (DC) Reichenbach). They provide a key to the sections in their subgenus *Aria* based on morphological characters, including foliar characters and on style and seed placentation. My opinion is that this provides a firm basis for the treatment of the wider group. However, my opinion is that most of their sections justify treatment at the genus level. So whereas Aldosoro *et al* treat *Chamaemespilus* as a section, the above review of published molecular data provides justification for its treatment as a genus, as followed by Roemer (1847) and Robertson *et al* (1992).

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[11] Fay & Christenhusz accept *Pyracantha* as a separate lineage based on an ill-defined paper attributed to Zhang *et al* [2017] which does not feature in their bibliography and is at variance with Campbell *et al* (2007), Potter *et al* (2007) and Lo & Donaghue (2012) where *Pyracantha* is often central in the cladograms. Fertile hybrids between *Pyracantha* and *S. commixta* (*XSorbocantha*) have occurred in cultivation.

Guo *et al* (2016) provide a cladogram based on nrITS DNA from 40 samples of *Sorbus s.l.* with three *Cotoneaster* and one each of *Eriobotrya* Lindley and *Pyracantha* as out-groups. The data as presented does not give any origins for the samples apart from that of the subject species named in the paper as *Sorbus calcicola* W. B. Liao & W. Guo. Thus there has to be a caveat about their conclusions. However, the presented cladogram, figure 1, is interesting. *Pyracantha* and the clade for the three *Cotoneaster* taxa are not resolved from the clade containing all the other samples—thus contradicting a basic tenet in Fay & Christenhusz (2018) and removing part of their justification for a super *Pyrus*. The clade containing the 13 *Sorbus s.s.* samples branches off from the clade containing the *Eriobotrya* sample and those for the other whitebeams. Next to separate is a clade containing "*Sorbus aria*", "*Sorbus graeca*" and "*S. aria* var. *salicifolia*"[12]. If this clade includes correctly identified *Sorbus aria* it fills the omission in Campbell *et al* (2007) where the *Aria* sample is *alnifolia* and provides support for treating *Aria* as a separate genus. Within the larger whitebeam clade, the two samples of *alnifolia* are centrally located. The figure provides good, but not total, support for the treatment proposed below.

Key to the genera in the Malinae

As suggested above, a drawback in Robertson *et al* is that they were not able to provide a key to the genera in their concept of the group. They provided written descriptions of the genera they accepted but not in a manner which makes it easy to compare them. My attempt to rectify this shortcoming is presented below. It is based on literature references, especially Robertson *et al*, and the study of plants in the wild and in cultivation. The Key recognizes 45 genera, including several new genera which are formally described below.

1	A	Leaf trace on shoot showing 5 sets of vascular bundles in petiole, leaves pinnate	2
	B	Leaf trace showing 3 sets of vascular bundles in petiole, leaves simple or pinnate	3
2	A	Fruits more than 18mm, cells of flesh filled with tannins, bark deeply fissured	*Cormus*
	B	Fruits less than 15mm, cells without or no more than a trace of tannins, bark not as above	*Sorbus*
3	A	Ovules 3—many per carpel	4
	B	Ovules 1 or 2 per carpel	7
4	A	Styles free at base, erupting through a pit in the top of the hypanthium	*Cydonia*
	B	Styles fused at base or in lower 1/3rd	5
5	A	Styles fused at base only, ovules 3—10 per locule, leaves evergreen or semi-evergreen, calyx persistent, fruit hairy	*Docynia*
	B	Styles fused in lower 1/3rd, ovules >10 per locule, leaves deciduous, calyx deciduous, fruit glabrous	6
6	A	Stamens 25 or less	*Pseudocydonia*
	B	Stamens 40—55	*Chaenomeles*
7	A	Leaves fully pinnate with leaflets of similar size, 1 ovule per carpel	*Osteomeles*
	B	Leaves simple, or with 1—3 free leaflets at base, 2 ovules per carpel, rarely just 1	8

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[12] This name is given as a synonym of the tetraploid *Aria rupicola* in Sennikov & Kurtto

8	A	Seeds PYRENES with an extremely hard or bony seed coat	9
	B	Seeds not PYRENES, seed coat leathery	15
9	A	Thin fleshy layer which can be scrapped off separating the Pyrenes	10
	B	No fleshy layer separating the Pyrenes	12
10	A	Leaf venation craspedodromus, stamens 5—20	*Crataegus*
	B	Leaf venation camptodromus, stamens 20—40	11
11	A	Flowers single, many sclereids in flesh of fruits, leaves deciduous, stamens 20—40	*Mespilus*
	B	Flowers in large panicles, sclereids lacking in fruit flesh, leaves evergreen, stamens 20	*Hesperomeles*
12	A	Carpels/styles 1	13
	B	Carpels/styles 2—5	14
13	A	Stamens 20, leaves entire, not toothed, no adnation of hypanthium to carpel	*Dichomanthes*
	B	Stamens 10—15, leaves toothed, nearly full adnation	*Chamaemeles*
14	A	Plants with thorns, leaves toothed	*Pyracantha*
	B	Plants thorn less, leaves entire	*Cotoneaster*
15	A	Leaves evergreen, persisting for more than one year	16
	B	Leaves deciduous, falling at least before next year's leaves flush	20
16	A	Calyx soon deciduous, leaving a round depression at top of fruit	*Raphiolepis*
	B	Calyx persisting in ripe fruit	17
17	A	Styles fused at least at base	18
	B	Styles free	19
18	A	Inflorescence compound corymb, fruit less than 10mm	*Photinia*
	B	Inflorescence paniculate, fruit 7—50mm	*Eriobotrya*
19	A	Styles 2, stamens 10, fruit core leathery & broad	*Heteromeles*
	B	Styles 2—5, stamens 10—20, fruit pseudoberry without leathery core	*Malacomeles*
20	A	Stamens 25—55, if less than 30 stamens then fruit densely lenticellate & diameter or length more than 20mm	21
	B	Stamens 15—20(—30), fruit dimensions less than 20mm or if larger then fruit elenticellate	22
21	A	Flowers in large leafless corymbose panicles which are terminal on leafless shoots in autumn, stamens (25—)30—50, ovules superposed, carpels 3—5(—7) ovary fully adnate to hypanthium, fruit 2.5—5cm densely lenticellate, calyx deciduous or incurved	*Pleiosorbus*
	B	Flowers in leafy inflorescences in spring, in umbels or umbellate racemes, stamens 50—55, ovules co-lateral, carpels 4—5, fruit c. 2.5cm, not densely lenticellate, calyx prominent, lobes long, ovary only ¾ adnate to the hypanthium	*Macromeles*

22	A	Leaf venation on flowering shoots craspedodromus with veins running to teeth or stopping a mm or so before the margin (hemi-craspedodromus) but not anastomosing	23
	B	Leaf venation on flowering shoots camptodromus with veins looped or curved forwards, not parallel, not or rarely running straight to teeth	36
23	A	Fruit noticeably longer than broad, rarely nearly round, carpels/styles 2(—3), ovules co-lateral	*Alniaria*
	B	Fruit round to oblate, not noticeably longer than broad or rarely longer than broad when the fruits/flowers are in umbels or umbel-like racemes, styles/carpels 2—5, ovules co-lateral or superposed	24
24	A	Calyx deciduous, usually well before fruit matures leaving a circular depression	25
	B	Calyx persistent in mature fruit	28
25	A	Leaves silvery hairy on lamina beneath with rufous hairs on veins, flowers/fruits in corymbs	*Dunnaria*
	B	Leaves glabrous or sparsely hairy beneath, flowers/fruits in umbels, umbel-like racemes or convex or domed panicles	26
26	A	Flowers/fruit in panicles or corymbose panicles, leaves serrate but not lobed	27
	B	Flowers/fruits in umbels or short umbel-like racemes < 0.5cm, leaves lobed, especially on vegetative shoots	*Sinomalus*
27	A	Ovules superposed	*Thomsonaria*
	B	Ovules co-lateral	*Micromeles*
28	A	Flowers in umbels or umbel-like short racemes less 1cm, styles/carpels 5	29
	B	Flowers in corymbs or corymbose panicles, styles/carpels 2—4	30
29	A	Leaves with 3 lobes extending more than 1/3rd way to midrib, lobes acute, lateral lobes often divided, fruit ripens red to yellow, not waxy	*Eriolobus*
	B	Leaves shallowly lobed only on vigorous extension shoots, generally lobulated or toothed, fruit ripens green, rarely yellow, with a waxy exudation	*Chloromeles*
30	A	Leaves with 3—5 distinct lobes extending > 1/3rd way to midrib, carpels 2, fruit brown with many close or contiguous lenticels	*Torminalis*
	B	Leaves unlobed or lobulated with lobes less than ¼ way to midrib	31
31	A	Styles free	32
	B	Styles fused at least at base	34
32	A	Leaves simple or doubly toothed but not lobed with lobes extending no more than 1/5th way to midrib, leaf underside silvery hairy	*Aria*[13]
	B	Leaves with some free leaflets at base or lobes extending more than 1/5th way to midrib, leaf undersides grey tomentose	33

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[13] The tree widely cultivated in the UK as *Sorbus* 'John Mitchell' keys out here. It is known to have been raised at Westonbirt from seeds of *Griffitharia vestita* and it would appear that the other parent was a species of *Karpatiosorbus*, perhaps *devoniensis* or *latifolia*.

33	A	Leaves with 1 or 2 (—3) free leaflets at base or pinnatilobed, fruit red to crimson	*Hedlundia*
	B	Leaves pinnatifid or sub-pinnate, fruit orange to red	*Borkhausenia*
34	A	Ovules co-lateral, styles 2—3, fruit yellowish-brown, orange-brown or reddish-brown with numerous large lenticels	*Karpatiosorbus*
	B	Ovules superposed, styles 2—4(—5), fruit brown, green to yellow, often pink tinged, lenticels dense to well spaced	35
35	A	Styles 3 or 4, leaves glabrous or sparsely hair when young, not tomentose, fruit brown, 12—20mm in diameter, covered in massed contiguous lenticels	*Wilsonaria*
	B	Styles 2—4(—5), leaves white to silver tomentose beneath, fruits green to yellow, often pink tinged, 7—15(—30)mm lenticels well spaced or absent	*Griffitharia*
36	A	Calyx persistent in fruit	37
	B	Calyx deciduous in fruit, usually dropping off to leave a circular scar before ripe	47
37	A	Pedicels & peduncles conspicuously warty or lenticellate, glabrous, pedicels long & slender, fruit without lenticels	*Pourthiaea*
	B	Pedicels & peduncles with few scattered smooth lenticels, usually hairy, fruit with lenticels	38
38	A	Flowers/fruits in umbels, umbel-like racemes or in long racemes	39
	B	Flowers/fruits in panicles or corymbose panicles	44
39	A	Flowers/fruits in racemes > 2cm, styles/carpels usually 5	*Amelanchier*
	B	Flowers/fruits in umbels or umbel-like racemes < 2cm, styles/carpels 2—5	40
40	A	Styles free	41
	B	Styles joined at least at base	42
41	A	Styles passing through a pit at top of hypanthium, stamens 15—30, flesh of fruit with large stone cells, 2 ovules in each carpel not separated by a false partition	*Pyrus*
	B	Styles not constrained by disk, stamens 20, flesh with few sclereids, ovules in carpels separated by a false partition	*Peraphyllum*
42	A	Ovules superposed, flowers/fruits in umbellate racemes, rachis 1—2cm	*Prameles*
	B	Ovules co-lateral, flowers/fruits in umbels or fascicles, rachis less than 1cm	43
43	A	Fruit fragrant, often waxy, dense layer of scelereid cells around the core & under the skin, flowers/fruits in umbel-like racemes, leaves often lobulated	*Chloromeles*
	B	Fruit not fragrant, few scelereid cells, flowers/fruits in umbels, rachis less than 5mm, leaf margin serrate but not lobulated	*Malus*
44	A	Petals pink or red, upright or erect, styles 2(—3), fruit red, >1cm	45
	B	Petals white or pink, spreading, styles 5, fruit < 1cm	*Aronia*
45	A	Leaves glabrous, leaf margin finely serrate	*Chamaemespilus*
	B	Leaves variously tomentose beneath, if glabrous then leaf margin doubly serrate or with small obtuse lobes	46

46	A	Leaves white tomentose beneath, margin minutely to doubly serrate	*Majovskya*
	B	Leaves glabrous to white or grey tomentose, margin doubly serrate or with very small obtuse lobes	*Normeyera*
47	A	Styles free, passing through a pit at the top of the hypanthium	*Pyrus*
	B	Styles joined at the base or in lower half	48
48	A	Flowers/fruit in panicles or corymbose panicles	49
	B	Flowers/fruits in umbels or umbel-like racemes with a rachis < 1cm	*Sinomalus*
49	A	Ovules superposed	*Thomsonaria*
	B	Ovules co-lateral	*Micromeles*

Proposed taxonomic treatment of *Sorbus sensu lata*.
Sorbus L. Sp. Pl. 2: 477. [1753]—Lectotype, designated by Rehder, 1949: *S. aucuparia* L. Two subgenera, 11 sections and circa 70—100 species, both diploid sexual and apomictic triploids and tetraploids. Distribution: Europe, North Africa (Morocco), Asia, North America. With *Cormus*, this genus is 'unique' in the leaf scar on the shoot showing five clusters of vascular tissue, whereas all the other genera in the Malinae have three.

Aria (Persoon) Host. Fl. Austriac. 2: 7 [1831]—Type *A. edulis* (Willdenov) M. Roemer Fam. Nat. Syn. Monogr. 3: 124. 1847. One (—few?) sexual diploid species and several tetraploid apomictic taxa, Europe, North Africa & West Asia.

Torminalis Medikus Philos. Bot. (Medikus) 1: 134. [1789] Type (only species listed by Medikus) *T. clusii* (M. Roemer) K. R. Robertson & J. B. Phipps. Syst. Bot. 16: 390. [1991] (= *Torminaria clusii* M. Roemer, Fam. Nat. Reg. Veg. Syn. 3: 130. [1847]—*Crataegus torminalis* L.). One species in Europe, North Africa & West Asia, second species, *T. orientalis* (Schonbeck-Temesy) K. R. Robertson & J. B. Phipps. Syst. Bot. 16: 390 [1991], in Iran.

Chamaemespilus Medikus Philos. Bot. (Medikus) 1: 138, 155. [1789] Type (only species listed by Medikus) *C. alpina* (Miller) K. R. Robertson & J. B. Phipps. Syst. Bot. 16: 390.—*Crataegus alpina* Miller, Gard. Dict., 8th ed., species number 3 under *Crataegus*. [1768]—*Mespilus chamaemespilus* L. Sp. Pl. 1: 479. [1753]. One species in Central Europe from Germany south to Spain, east to Greece and north to Bulgaria.

European and West Asian hybrid species involving *Aria edulis* (all) with variously *Sorbus aucuparia*, *Torminalis clusii* and rarely *Chamaemespilus alpina* are treated in Sennikov & Kurtto (2017) under the genera ***Hedlundia*** Sennikov & Kurtto (*Aria x Sorbus*), ***Borkhausenia*** Sennikov & Kurtto (*Aria x Sorbus x Torminalis*), ***Karpatiosorbus*** Sennikov & Kurtto (*Aria x Torminalis*), ***Majovskya*** Sennikov & Kurtto (*Aria x Chamaemespilus*) and ***Normeyera*** Sennikov & Kurtto (*Aria x Chamaemespilus x Sorbus*).

Cormus Spach Hist. Nat. Veg. (Spach) 2: 96. [1834] Type (only species listed by Spach) *C. domestica* (L.) Spach Hist. Nat. Veg. (Spach) 2: 97. [1834]—*Sorbus domestica* L. Sp. Pl. 477. 1753. One species in Europe, North Africa and West Asia to the Caucasus. There are no recorded hybrids of this genus.

Micromeles Decaisne Nouv. Arch. Mus. Paris 10: 168. [1874] Lectotype, designated by H. Ohashi & H. Iketani, J. Jap. Bot. 68: 357. [1993] *M. rhamnoides* Decaisne, loc. cit. 169. Robertson *et al* proposed *Sorbus alnifolia* as lectotype but this has to be rejected as Decaisne [1874] placed this species in *Aria* and

specifically excluded it from *Micromeles*. Aldosoro *et al* (p 170) proposed *M. griffithii* Decaisne having overlooked Ohashi & Iketani. This lectotype is superfluous.
The genus is characterised by the small, 4—7mm fruits without lenticels, two co-lateral ovules in the two carpels and generally craspedodromus venation, but camptodromus in *M. cuspidata.*

Decaisne established *Micromeles* with five species, viz. *verrucosa, castaneifolia, rhamnoides, khasiana* and *griffithii*[14]. *M. verruculosa* is based on the same collections (although probably not exactly the same specimens) as the earlier described *Pyrus cuspidata* Bertoloni. *M. castaneifolia* is based on Griffith 2077 which at Kew appears to fit *cuspidata*, although Aldosoro *et al* cite it as synonymous with *Pyrus granulosa* Bertoloni. Hooker f. (1878, Flora of British India 2: 379) has *castaneifolia* as a 'doubtful species'. *M. khasiana* also is often treated as a synonym of *granulosa*, with which it shares a larger—circa 10—12mm) 4 celled fruit with a granular pulp. My view is that *M. granulosa* and *M. khasiana* do not fit in *Micromeles* but as part of the new genus *Thomsonaria* as proposed below. Thus on current information, of Decaisne's five species, I would only include *M. rhamnoides*, *M. griffithii* and *P. cuspidata* in his genus. To this I provisionally add *Pyrus polycarpa* Hooker f., *Sorbus paucinerva* Merrill and *Sorbus salwinensis* T. T. Yu & L. T. Lu. Accordingly this requires the following new combinations:

Micromeles cuspidata (Bertol.) Rushforth. **Comb. nov.** —*Pyrus cuspidata* Bertol. Mem. Reale Accad. Sci. Ist. Bologna, ser. 2, 4: 311 [1864]. More commonly know from the later synonym based on *Micromeles verrucosa* Decaisne which has priority only in the genus *Sorbus*. It was described from the Khasia Hills, Meghalaya State, India. I am unclear as to how far east the true species is found as the name has been applied to various quite different entities, e.g. see *Thomsonaria subulata,* which has a much larger lenticellate and granular fruit with superposed ovules. I suspect that it is from such specimens that Aldosoro *et al* record *Pyrus cuspidata* [as *Sorbus verrucosa*] as having superposed ovules.

Micromeles paucinerva (Merrill) Rushforth. **Comb. nov.** —*Sorbus paucinerva* Merrill. Brittonia 4: 75 [1941]. This species was found by Kingdon-Ward as part of the Vernay-Cutting expedition. It was only collected in flower but seems to have a relationship with *T. cuspidata.*

Micromeles salwinensis (T. T. Yu & L. T. Lu) Rushforth. C**omb. nov.**—*Sorbus salwinensis* T. T. Yu & L. T. Lu, Acta. Phytotax. Sin. 13(1): 102 [1975]. Kingdon-Ward 12955, 13054, 13499, 20890 and 21377, all collections from Burma, appear to belong to *M. salwinensis*, thus extending the range of *M. salwinensis.*

Micromeles polycarpa (Hooker f.) Rushforth. **Comb. nov.**—*Pyrus polycarpa* Hook. f., Fl. Brit. India 2: 378. [1878].

Pleiosorbus Zhou LiHua & Wu ZhengYi Act. Bot. Yunn. 24(4): 383—389 [2000]. Holotype *P. megacarpus* Zhou & Wu. This well marked genus is characterised by the superposed ovules in the large (2.5—5cm) extremely strongly lenticellate fruits. The flowers are in large terminal clusters on leafless shoots and open in the autumn, with the fruits ripening a year later. The flowers have upwards of (25—)32—50 stamens, with 3—5(rarely to seven) styles and carpels with generally 2 ovules. In *Sorbus* in Flora of China vol 9, p 122 (Lu & Spongberg, 2003) it is reported that the ovules are (2—)3(—4) in each carpel but fruits I have opened have only had 2 ovules. The genus was established from collections made in the Medog region of South East Tibet collected in 1992. However, in May 1926 Kingdon-Ward found much the same plant in the Seinghku valley in the far north of Burma. Kingdon-Ward's specimens were named as *Eriobotrya wardii* C. E. C. Fischer. Later in 1935/37 (see Kingdon-Ward [1941] page 266) he saw it again in the forest around Lagam, West Kameng district, Arunachal Pradesh, India. The genus has since been seen in Mechuka (West Siang district, Arunachal Pradesh), East Siang district and on the southern flank of Madoi Razi which is due north of PutaO, Kachin State, Burma. Examination shows that specimens from Burma differ from the material from Medog, Mechuka and Lagam in having a persistent calyx, cf. a deciduous calyx leaving a circular depression (see photograph 94 in Sun Hang & Zhou ZheKun [2000]). The fruit character of a persistent calyx is not recorded from the west side of the Indo-Burman range. Accordingly with our present knowledge of the genus, I propose to treat the genus as having two species, with *P. megacarpus* for the material from South East Tibet and North East India and *P. wardii* from Burma, requiring the following new combination:

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[14] Decaisne spelt it as *griffithsii* but this is a typographical error universally corrected in literature.

Pleiosorbus wardii (C. E. C. Fischer) Rushforth. **Comb. nov.**—*Eriobotrya wardii* C. E. C. Fisher, Bull. Misc. Inform. Kew 1929(6): 205 [1929].

Griffitharia Rushforth. **Genus nov**. Type *Griffitharia guanii* (Rushforth) Rushforth.
This genus is distinguished by the craspedodromus venation to the leaves which are densely tomentose adaxially (only cobwebby in *G. wardii* and, nearly glabrous in early growth leaves in *G. needhamii*): the leaf margin is serrate or somewhat doubly serrate, but shallowly lobulated in *G. lanata.* The leaf hairs are generally white or silvery white on the lamina but in some species those on the veins are rufous. The fruit is 7—20(—30) mm, ovoid, oblate or rarely ellipsoidal. It is green, often with a pink or purplish tinge, and ripens to russet or yellow but not red. The flowers/fruits have 2—5 styles and carpels and 20 stamens. The styles are joined at the base and pressed together in the lower third. The two ovules in each carpel are superposed and the seeds radially inserted. The calyx is persistent with somewhat fleshy lobes. The hypanthium is nearly fully adnate to the ovary. The disk is epigynous. The fruits are moderately to heavily lenticellate except in *G. lanata* and *G. thibetica* where they are elenticellate. The carpels are inserted into the flesh off a central axis and the flesh rather granulose. The seeds are rounded to oboval, about half as thick as wide with rather acute angled margins, and thus resemble the seeds of apples (*Malus*) with persistent calyces.

Griffitharia is primarily Sino-Himalayan in distribution, from Eastern Afghanistan in the west along the Himalayan axis to West and Central China. The species are typical of the cool temperate zone, from 1850m to about 3300m and form small to medium trees, rarely growing to more than 20m. Generally the species are vicariads, although two species may be found in the same area but at different altitudes or perhaps aspects.

The following new combinations are required:
Griffitharia guanii (Rushforth) Rushforth. **Comb. nov.**—*Sorbus guanii* Rushforth, Int. Dendrol. Soc. Year Book 2009: 88 [2010]. The species is recorded from Huadianba at the northern end of the Cang shan, Yunnan.

Griffitharia vestita (Wall. ex G. Don) Rushforth. **Comb. nov.** —*Pyrus vestita* Wall. ex G. Don, Gen. hist. 2: 647 [1832]. This species occurs from Central/West Nepal to North West India.

Griffitharia sharmae (M. Watson, V. Manandhar & Rushforth) Rushforth. **Comb. nov.** —*Sorbus sharmae* M. Watson, V. Manandhar & Rushforth, Int. Dendrol. Soc. Year Book 2009: 79 [2010]. This species is found in Central to East Nepal.

Griffitharia hedlundii (C. K. Schneider) Rushforth. **Comb. nov.** —*Sorbus hedlundii* C. K. Schneider, Ill. Handb. Laubholzk. 1: 685 [1906]. This species is recorded from Southern Sikkim. The plants in East Nepal with similar rufous hairs differ in the more strongly toothed leaves. Plants similar to *G. hedlundii* are recorded from Central South Bhutan (around Sengor) and perhaps east into Arunachal Pradesh.

Griffitharia karchungii (Rushforth) Rushforth. **Comb. nov.** —*Sorbus karchungii* Rushforth, Int. Dendrol. Soc. Year Book 2009: 81 [2010]. This is the species found in Bhutan. The type is from the higher altitude form. In central Bhutan at lower altitude the trees have large leaves but otherwise seem to fit here.

Griffitharia heseltinei (Rushforth) Rushforth. **Comb. nov.** —*Sorbus heseltinei* Rushforth, Int. Dendrol. Soc. Year Book 2009: 83 [2010]. This tree comes from the Tongkyuk and Gyala districts of SE Tibet to the north of the main Himalaya axis. The generally obovoid fruit has a distinctly yellow flesh, similar to the external colour.

Griffitharia heseltinei var. *glabrescens* (T. T. Yu & L. T. Lu) Rushforth. **Comb. nov.** —*Sorbus coronata* var. *glabrescens* T. T. Lu & L. T. Lu, Acta. Phytotax. Sin. 18: 494 [1980]. The recorded localities are Medog and Showa districts in SE Tibet and thus from the south and east of the record for the species; these are mainly on the south side of the main Himalaya axis. It differs from the species in the more glabrescent underside to the somewhat larger foliage. On present evidence it appears to be only a minor variant, hence the retention of the varietas status (rather than as subspecies).

Griffitharia wardii (Merrill) Rushforth. **Comb. nov.** —*Sorbus wardii* Merrill, Brittonia 4: 75 [1941]. This species is frequently cited in synonymy under *G. thibetica.* However, it differs from this species in the presence of lenticels in the fruit and smaller leaves. It is also easily separated by the character of the abaxial surface of the leaf. The covering of hairs is never dense and reveals the 'bullulate' under surface, with small raised lumps separated by dips—this character is always present in *G. wardii* but is occasionally found in *G. karchungii* and *G. schwerinii.* The species occurs from the Arunachal Pradesh border with Bhutan east to Northern Burma and the Dulong region of Yunnan, being a component of the upper temperate zone. Aldosoro *et al* propose the Kew specimen of KW 9623 as lectotype but Merrill was quite specific in Brittonia [1941] that unless specimens were noted as elsewhere, the holotypes were at the Arnold Arboretum (HUH), which is where the holotype of *Griffitharia wardii* resides.

Griffitharia burtonsmithiorum (Rushforth) Rushforth. **Comb. nov.** —*Sorbus burtonsmithiorum* [as *burtonsmithii*] Rushforth, Int. Dendrol. Soc. Year Book 2009: 85 [2010]. This is found in Northern Burma and the Dulong district of Yunnan at 1850—2700m, below the level at which *G. wardii* occurs.

Griffitharia thibetica (Cardot) Rushforth. **Comb. nov.** —*Pyrus* [as *Pirus*] *thibetica* Cardot, Notul. Syst. (Paris) 3: 349 [1918]. The name, as *Sorbus thibetica* (Cardot) Hand.-Mazz., is widely used to indicate any higher elevation species in this group from Yunnan to Bhutan and Nepal. Cardot's description, based on the type Soulie 1237 from Thra-na near Tsekou in the Mekong valley, Yunnan, describes the fruit as "*haud lenticellati*", i.e. without lenticels. This feature shows clearly in two George Forrest collections from the same place but is not otherwise shown by any other specimens from elsewhere in Yunnan, Burma, NE India or Bhutan which are all characterised by the many, and frequently large, lenticels on the fruit. The evidence indicates that this species is a local endemic in the valleys to the west of Tsekou which are known for local endemics, such as the only habitat for *Pseudotsuga forrestii* Craib.

Griffitharia atrosanguinea (T. T. Yu & Tsai) Rushforth. **Comb. nov.** —*Sorbus atrosanguinea* T. T. Yu & Tsai, Bull. Fan Mem. Inst. Biol. (Bot) 7: 119 [1936]. This is the common upland species in the genus from Western Yunnan. Apart from the strongly lenticellate fruit, the leaves are narrower than in *G. thibetica*, which may explain how Aldosoro *et al* confused it with *G. hemsleyi.*

Griffitharia hudsonii (Rushforth) Rushforth. **Comb. nov.** —*Sorbus hudsonii* Rushforth, Int. Dendrol. Soc. Year Book 2009: 89 [2010]. This tree is recorded from South West Yunnan from Lincang Daxue shan (Hunhua shan) and from the border of Yunnan with Burma as far north as 26^0 30' N.

Griffitharia yongdeensis (Rushforth) Rushforth. **Comb. nov.** —*Sorbus yondeensis* Rushforth, Int. Dendrol. Soc. Year Book 2009: 90 [2010]. The original spelling as *yondeensis* is a typographical error for Yongde shan and I take this opportunity to correct it. Currently *G. yongdeensis* is only known from the Yongde DaXue shan, 24^0 07' 17" N, 99^0 39' 19" E, above Wu Mu Long township in South West Yunnan. *G.*

yongdeensis has *G. hudsonii* a short distance to the South East and to the North West, but the size, shape and texture of the foliage easily separates them.

Griffitharia pallescens (Rehder) Rushforth. **Comb. nov.** —*Sorbus pallescens* Rehder, Plantae Wilsonianae 2: 266 [1915]. This species is restricted to the area close to the West and South West of Kangding (Tachien-lu) in West Sichuan. It is characterised by the small—less than 5cm—ovate to ovate-oblong leaves on the flowering spur shoots and the small trusses of circa 15 flowers; leaves on extension shoots may be larger. The name, as *S. pallescens*, is commonly mis-used for the much more widely distributed species in South West Sichuan and across Yunnan, here treated as *G. ambrozyana*, which differs in the larger oblong to lanceolate leaves and larger trusses.

Griffitharia ambrozyana (C. K. Schneider) Rushforth. **Comb. nov.** —*Sorbus ambrozyana* C. K. Schneider, Bot. gaz. 63: 401 [1917]. **Syn. nov**. *Pyrus* [as *Pirus*] *coronata* Cardot. Notul. Syst. 3: 348 [1918]. This species is widespread across Yunnan and into South West Sichuan, such as the Muli district. The type specimen of Schneider 3913 at Harvard [HUH] has an extension shoot with lanceolate to narrow elliptic leaves contrasting with the oblong-ovate to oblong leaves in the fertile shoot. Cultivated plants often show this dimorphism between mature foliage and extension growths. Consequently I conclude that it is no longer appropriate to maintain the two as separate taxa.

Griffitharia needhamii (Rushforth) Rushforth. **Comb. nov.** —*Sorbus needhamii* Rushforth, Bot. Mag. 27 (4): 378 [2010]. Currently this species is only recorded from Leigong shan, Guizhou. The most distinctive aspect of this tree is the foliage; in the first flush, and thus what will be seen in a dried flowering or fruiting specimen, are almost glabrous leaves which are much closer in superficial appearance to the foliage of *Alniaria alnifolia*—only the later leaves show the hairy abaxial side typical of the genus but then the globose to oblate fruit with the persistent calyx crown will be present showing that the species belong here and not in *Alniaria*.

Griffitharia spongbergii (Rushforth) Rushforth. **Comb. nov.** —*Sorbus spongbergii* Rushforth, Int. Dendrol. Soc. Year Book 2009: 96 [2010]. This is recorded from southern Sichuan to the vicinity of Lijiang in Yunnan. It is best thought of as a southern vicariant of *G. pallescens*, differing in the larger inflorescence and larger and noticeably rufous hairy covering the veins on the underside of the leaves.

Griffitharia hemsleyi (C. K. Schneider) Rushforth. **Comb. nov.** —*Micromeles hemsleyi* C. K. Schneider, Ill. Handb. Laubholzk. 1: 704 [1906]. This species is found in North West Hubei west across to North Chongqing and Northern Sichuan to the Tsinling shan in Shaanxi. The unique feature is the long pointed vegetative buds.

Griffitharia schwerinii (C. K. Schneider) Rushforth. **Comb. nov.** —*Micromeles schwerinii* C. K. Schneider, Ill. Handb. Laubholzk. 1: 702 [1906]. Syn. *Sorbus henryi* Rehder, Plantae Wilsonianae 2: 276 [1915]. Schneider based this species on two specimens, Henry 8957 from the Emei shan (collected when Henry sent his most competent collector with Antwerp Pratt to West Sichuan) and Giraldi 986 from Tai Bai shan, Shaanxi. When Rehder transferred the species to *Sorbus* he could not make the obvious combination as Schneider had named a rowan—now generally treated as a synonym of *S. gracilis* (Sieb. & Zucc.) K. Koch—as *Sorbus schwerinii*. He provided a new name and effectively lectotypified the species on the Henry 8957 flowering specimen at Harvard (HUH). This lectotype is clearly consistent with the protologue, with only a few words in the description specific to the fruits. I have not seen the Giraldi 986 isotype but cultivated material from Tai Bai shan and southern Shaanxi does not match material in herbaria, on the Emei shan or in cultivation from the area around the Emei shan which is the area from which the Henry type was collected and to which I consider the species is restrict.

Griffitharia lanata (D. Don) Rushforth. **Comb. nov.** —*Pyrus lanata* D. Don, Prodr. Fl. Nepal. 237 [1825]. The species is recorded from West Nepal to Pakistan. It is odd for the genus in the lobulated leaves and in the generally larger fruits which are elenticellate.

In the cladogram in Guo *et al G. hemsleyi, G. thibetica, G. ambrozyana* (as *S. coronata*) and *G. pallescens* come out in one clade but with a subclade which includes *G. pallescens* as well as *Sorbus tsinlingensis* and *Sorbus calcicola*. I have not seen the type of *S. tsinlingensis* but the description in *Sorbus* in Flora of China vol 9: 124 (Lu & Spongberg, 2003) strongly supports treating it as part of the genus *Alniaria* until further information is available, especially as Guo *et al* do not indicate the source for their samples. Regarding *S. calcicola*, the photographs and line drawings suggest it is not a *Griffitharia* species. The combination of camptodromus venation, co-lateral ovules (as suggested by drawing C in fig. 2) and a persistent calyx are characters of *Pourthiaea* Decaisne [1874]. A key to the species of *Griffitharia* is provided in Rushforth [2010].

Alniaria Rushforth **Genus nov.** Type species *Alniaria alnifolia* (Sieb. & Zucc.) Rushforth.
This genus is characterised by the craspedodromus venation, the fruit which is basically longer than broad, rarely nearly as long as broad, the two carpels each with two co-lateral ovules and set in a well-developed core. The styles are joined at the base. The epigynous calyx is deciduous in most species but retained in *A. zahlbruckneri* and *A. hunanica* which have fleshy calyx lobes. The seeds are obovoid, rounded in transverse section with a rounded to mucronate tip, generally 6—7 mm by 3—4 mm and not flattened (if full) as found in *Griffitharia, Thomsonaria* and genera such as *Malus*. The fruit, with its co-lateral ovules and rounded seeds, shows a similarity to the (generally globose) fruits of *Sinomalus* but this has flowers/fruits in umbels.

The following new combinations are required:
Alniaria alnifolia (Sieb. & Zucc.) Rushforth. **Comb. nov.**—*Crataegus alnifolia* Sieb. & Zucc. Abh. Math.-Phys. Cl. Konigl. Bayer. Acad. Wiss. 4(2): 130 [1845]. This tree is found from Japan and Korea across Northern China to Gansu.

Alniaria japonica (Sieb.) Rushforth. **Comb. nov.**—*Sorbus japonica* Sieb. Verh. Batav. Genootsch. Kunst. Xii: 67 [1830]. This tree is recorded from Japan and Korea.

Alniaria zahlbruckneri (C. K. Schneid.) Rushforth. **Comb. nov.**—*Micromeles zahlbruckneri* C. K. Schneid. Bull. Herb. Boissier ser. II vi: 318 [1906]. It is recorded across central Northern China and possibly further south, and is unusual for the persistent calyx.

Alniaria folgneri (C. K. Schneid.) Rushforth. **Comb. nov.**—*Micromeles folgneri* C. K. Schneid. Bull. Herb. Boissier ser. II vi: 318 [1906]. This species is from central Northern China.

Alniaria nubium (Hand.-Mazz.) Rushforth. **Comb. nov.**—*Sorbus nubium* Hand.-Mazz. Anz. Akad. Wiss. Wien. Math.-Naturewiss Kl. 1921 lviii: 147 [1921]. This species has broader leaves than *A. folgneri* and is recorded from Hunan, Jiangxi and southern Anhui provinces, China.

Alniaria tsinlingensis (C. L. Tang) Rushforth. **Comb. nov.**—*Sorbus tsinlingensis* C. L. Tang. Fl. Tsinling. 1(2): 608 [1974]. This is recorded from Tai Bai shan, South Shaanxi and from SE Gansu. I have not seen material but the description of the fruit indicates that it belong here. Material of the tree at the Arnold Arboretum and recorded as grown from seeds sent from Beijing in 1988 does not belong with the species described in the Flora of China Rosaceae 9: 124 (Lu & Spongberg, 2003). It appears to belong with or be close to *Griffitharia hemsleyi*, which is also consistent with its position in the cladogram in Guo *et al*.

Alniaria chengii (C. J. Qi) Rushforth. **Comb. nov.**—*Sorbus chengii* C. J. Qi. J. Nanjing Technol. Coll. Forest Prod. 1981(3): 124 [1981]. I have not seen verified material of this species named from Hunan but a small grove on the Jiangxi side of Nan Feng Mian shan, the highest peak on the Hunan—Jiangxi border, appears to fit here.

Alniaria hunanica (C. J. Qi) Rushforth. **Comb. nov.**—*Sorbus hunanica* C. J. Qi. J. Nanjing Technol. Coll. Forest Prod. 1981(3): 125 [1981]. It is listed as a synonym of *A. zahlbruckneri* in Flora of China 9: 118 (Lu & Spongberg, 2003), but the fruits and foliage seem sufficiently distinct to warrant treating it as a separate species.

Alniaria yuana (Spongberg) Rushforth. **Comb. nov.**—*Sorbus yuana* Spongberg. J. Arnold Arbor. 67: 257 [1986]. This is a rare species from West Hubei and adjacent Chongqing provinces.
The cladogram in Guo *et al* (2016) has *A. alnifolia, A. folgneri, A. yuana* and *A. zahlbruckneri* clustered together. However, it also has *Dunnaria dunnii* (Rehder) Rushforth beside *A. folgneri* with *A. japonica* and *A. tsinlingensis* elsewhere. Accordingly their sampling provides significant but not total support for the above treatment.

Thomsonaria Rushforth. **Genus nov.** Type species *Thomsonaria thomsonii* (King ex Hook. f.) Rushforth. This genus is characterised by the combination of the two superposed ovules in each of the 2—4 carpels, the medium-sized fruits (0.7—1.2cm, rarely larger) which have a deciduous calyx which leaves a large circular scar (often with a conical centre from the fused tops of the ovary or base of the joined styles). The typical fruit is green or greenish with spaced lenticels but in some species it is brownish with many lenticels. The flesh is somewhat granulose. The seeds are similar to *Griffitharia.* The flowers have 20 stamens, rarely 15. The leaves are either camptodromus – the typical state – or craspedodromus. They vary from glabrous to initially densely hairy – where the indumentum is often floccose – but are not persistently tomentose on the abaxial surface as characterises the typical state in *Griffitharia* and some species in *Alniaria.* The species are found from the Himalaya east across China and south into Southeast Asia and Sumatra. They occur in warm to cold temperate zones.

Thomsonaria thomsonii (King ex Hook. f.) Rushforth. **Comb. nov.**—*Pyrus thomsonii* King ex Hook. f., Fl. Brit. India 2(5): 379 [1878]. Aldosoro *et al* record that Gabrielian has proposed Hooker f. s.n. Sikkim: 8000 feet [K] as lectotype, which is a flowering specimen. However, Hooker ascribed the name to King MSS, i.e. to King's manuscript. The protologue for *Pyrus thomsonii* reads: "King MSS. ; glabrous, leaves very shortly petiole, elliptic-lanceolate or –oblanceolate acuminate, serrate above the middle glabrous, styles 2—4, fruit ¾ in. diam." which makes no mention (apart possibly in the style number!) to a flowering specimen. In the Kew herbarium King 3046, reference K000758226, collected 16/9/1876, is a fruiting specimen which is annotated *Pyrus thomsonii* King in G. King's handwriting. Furthermore, as Hooker points out (1878, p 379) "Young flowering specimens of this were distributed as *Photinia arguta*, Wall., var. ?, and are cited by Decaisne under his *Micromeles verrucosa* (Pyrus cuspidata, Bertol.) from which it differs in the glabrous narrow leaves with short petioles serrated only beyond the middle." Taking these two points together I consider there is a strong case for rejecting Gabrielian's choice of lectotype in favour of King 3046, which I propose as the lectotype. Plants with elliptic-lanceolate or elliptic-oblanceolate leaves appear to be restricted to the Sikkim area. In West Kameng, Arunachal Pradesh the leaves are shorter and more elliptic than lanceolate. The Flora of China account (Lu & Spongberg, 2003) has it as coming from Burma, Yunnan and Sichuan, a range extension which I do not believe is likely. The leaf drawing, from Forrest 13390 shown as *thomsonii* in Fig. 24A in Aldosoro *et al* suggests a different species than the Sikkim one.

Thomsonaria ferruginea (Hook. f.) Rushforth. **Comb. nov.**—*Pyrus ferruginea* Hook. f., Fl. Brit. India 2(5): 379 [1878]. This is recorded from Bhutan and possibly elsewhere along the Eastern Himalaya.

Thomsonaria aronioides (Rehder) Rushforth. **Comb. nov.** —*Sorbus aronioides* Rehder. Plantae Wilsonianae 2: 268 [1915]. This was named from West Sichuan but may also extend along the Yunnan—Burma border region.

Thomsonaria globosa (T. T. Yu & Tsai) Rushforth. **Comb. nov.** —*Sorbus globosa* T. T. Yu & Tsai. Fan Mem. Inst. Biol. Bot. 7: 121 [1936]. This was named from West Yunnan. The Flora of China account (Lu & Spongberg, 2003) includes adjacent Burma but also gives it as being found in Guizhou and Guangdong provinces. Some DNA investigation covering the full range might be interesting.

Thomsonaria ligustrifolia (A. Chevalier) Rushforth. **Comb. nov.** —*Pyrus ligustrifolia* A. Chevalier. Rev. Int. Bot. Appl. Agric. Trop. 22: 375 [1942]. Named from Fansipan in North Vietnam, this small tree may also occur in Southern Yunnan.

Thomsonaria verticillata (Merrill) Rushforth. **Comb. nov.** —*Sorbus verticillata* Merrill. Brittonia 4: 77 [1941]. First named from the Adong valley in Kachin State, Burma, it has since been found in far eastern Arunachal Pradesh State, India.

Thomsonaria caloneura (Stapf) Rushforth. **Comb. nov.** —*Micromeles caloneura* Stapf. Bull. Misc. Inform. Kew 1910: 192 [1910]. This is a tree found over a wide area of Central China, mainly as subsp. *kwangtungensis* (T. T. Yu) Rushforth **Comb. & stat. nov.**—*Sorbus caloneura* var. *kwangtungensis* T. T. Yu, Acta Phytotax. Sin. 8: 223 [1963], which has slightly fewer veins and less coarsely toothed leaf margins.

Thomsonaria meliosmifolia (Rehder) Rushforth. **Comb. nov.** —*Sorbus meliosmifolia* Rehder. Plantae Wilsonianae 2: 270 [1915]. This was first named from West Sichuan but is now recorded from adjacent areas of Yunnan and Guizhou.

Thomsonaria elenorae (Aldosoro, Aedo & C. Navarro) Rushforth. **Comb. nov.** —*Sorbus elenorae* Aldosoro, Aedo & C. Navarro. Syst. Bot. Mono. 69: 51 [2004]. This species is a constituent of the warm temperate mountain flora from Guangdong, Guangxi, Guizhou, Hunan and Jiangxi. Aldosoro *et al* cite a specimen from Henan, which is unlikely to be a correct identification from so much further north; however, as the cited collection is numbered "Guangdong team 5280" I suspect a typographical error.
This species has been mis-identified in the Flora of China 9: 117 (Lu & Spongberg, 2003) as part of the variation of *Sorbus megalocarpa* Rehder (here treated as *Wilsonaria megalocarpa*, see below) and the range given for *W. megalocarpa* as including Guizhou, Guangxi, Hunan and Jiangxi probably refers to *T. elenorae*.

Thomsonaria decaisneana (C. K. Schneid.) Rushforth. **Comb. nov.** —*Micromeles decaisneana* C. K. Schneid. Bull. Herb. Boissier, ser. 2, (vi): 269 [1906]. Synonym *Micromeles keissleri* C. K. Schneider, *Sorbus keissleri* (C. K. Schneid.) Rehder. This tree is recorded in Central China from West Hubei, Sichuan, Hunan, Guizhou, Jiangxi and Guangxi. It is more commonly known under the *keissleri* synonym as in *Sorbus* there is an earlier combination *Sorbus decaisneana* (which is a synonym of *Aria edulis*). However, in any other genus the *decaisneana* name has priority.

The following six species (*T. granulosa, T. corymbifera, T. khasiana, T. malayensis, T. crenulata* and *T. fragrans*) have traditionally been treated as one wide ranging taxon from the Khasia Hills of Meghalaya to Sumatra and north to North Vietnam and southern China. From 1915 this was under the name *Sorbus granulosa* (Bertol.) Rehder but since 1973 when Miquel's *Vaccinium corymbiferum* was identified as belonging in this group then either as *Micromeles* or *Sorbus corymbifera*. As yet I have not critically examined material from across the range but less detailed examination suggests that several, if not all of them, are not the same taxon and accordingly that it is appropriate to make all six new combinations. In particular, I note that Meijer 3460 from Mt. Merapi in Central Sumatra [K] has only 15 stamens whereas the flowering specimens from other localities appear to have 20 stamens. Clearly more investigation is required, including consideration of Spongberg's comment (Flora of China 9: 122, (Lu & Spongberg, 2003)) where he expresses the opinion that the variation between *T. corymbifera sensu lata* and *T. meliosmifolia* is continuous.

Thomsonaria granulosa (Bertol.) Rushforth. **Comb. nov.** — *Pyrus granulosa* Bertol. Mem. Reale Accad. Sci. Ist. Bologna, ser. 2, 4: 312 [1864]. This species is named from the Khasia Hills, Meghalaya, India.

Thomsonaria corymbifera (Miq.) Rushforth. **Comb. nov.** —*Vaccinium corymbiferum* Miquel. Fl. Ned. Ind. Eerste bijv.: 558 [1861]. This species is named from Sumatra, Indonesia.

Thomsonaria khasiana (Decaisne) Rushforth. **Comb. nov.** —*Micromeles khasiana* Decaisne. Nouv. Arch. Mus. Paris 10: 168. [1874]. This species is named from the Khasia Hills.

Thomsonaria malayensis (Ridley) Rushforth. **Comb. nov.** —*Micromeles malayensis* Ridley. J. Bot. 62: 296 [1924]. This species is named from Peninsular Malaya.

Thomsonaria crenulata (E. T. Geddes) Rushforth. **Comb. nov.** —*Pyrus crenulata* E. T. Geddes. Bull. Misc. Inform. Kew 1929: 108 [1929]. This species is named from Thailand.

Thomsonaria fragrans (E. T. Geddes) Rushforth. **Comb. nov.** —*Pyrus fragrans* E. T. Geddes. Bull. Misc. Inform. Kew 1930 (4): 161 [1930]. This species is named from Thailand.

Thomsonaria subulata (J. E. Vidal) Rushforth. **Comb. nov.** —*Sorbus verrucosa* Decaisne var. *subulata* J. E. Vidal. Fl. Cambodge, Laos, Vietnam 6: 32 [1968]; *Sorbus subulata* (J. E. Vidal) Ngyuen T. H. & Yakovlev. Bot. Zhurn. (Moscow & Leningrad) 66: 1188 [1981]. This species is named from Fansipan in Lao Cai province, VietNam.

Thomsonaria brevipetiolata (Nguyen T. H. & Yakovlev) Rushforth. **Comb. nov.** —*Sorbus brevipetiolata* Nguyen T. H. & Yalovlev Bot. Zhurn. (Moscow & Leningrad) 66: 1189 [1981]. This species is recorded from North Vietnam. It clearly does not have any close relationship with *Micromeles rhamnoides*, despite Aldosoro *et al* treating it as a synonym of this species.

Thomsonaria astateria (Cardot) Rushforth. **Comb. nov.** —*Pyrus* [as *Pirus*] *asterteria* Cardot. Lecomte, Not. Syst. 3: 348. This species is known from central Yunnan. Cardot based it on five specimens collected by Ducloux (one with Bodinier) and one by Delavay which are housed in the Paris herbarium. When Aldosoro *et al* were seeking these prior to 2004 they failed to locate them and proposed a neotype from the 1984 SABE expedition to the Cang shan in West Yunnan. However, when the Curator of the Edinburgh Herbarium asked to the specimens on loan in 2009 they were promptly sent to Edinburgh—perhaps as they were sought under Cardot's name? Four of the Ducloux specimens (Ducloux & Bodinier 0114, Ducloux 3318, 4115 and 4751) have very similar densely rufous hairy new leaves and flowers and are dated February to March. The Delavay specimen has larger better developed leaves and is in flower, although with the date as May 1890. The fifth Ducloux specimen, Ducloux 3485, is dated 28th May 1905 and is in immature fruit.

I propose Ducloux 4115, Paris reference number P00689928, as LECTOTYPE. This is one of the young flowering specimens, but crucially it has a note in Ducloux's writing linking it with Ducloux 3485. Whilst this is not proof that the two specimens were from the same tree, it indicates Ducloux's opinion that they were the same entity. Ducloux 4115 was collected from near to Kunming and all the material enumerated by Cardot is from Kunming west to the Cang shan.

Thomsonaria epidendron (Hand.-Mazz.) Rushforth. **Comb. nov.** —*Sorbus epidendron* Hand.-Mazz. Symb. Sin. 7: 466 [1933]. This tree, or large epiphytic shrub, was named from the Yunnan-Burma border. *Thomsonaria detergibilis* (Merrill) Rushforth. **Comb. nov.** —*Sorbus detergibilis* Merrill. Brittonia 4: 76 [1941]. Merrill based this species on material collected by Kingdon-Ward in the Adong valley, northern Kachin State, Burma.

T. asterteria, T. epidendron and *T. detergibilis* clearly show affinity to each other but come from different habitats and on the present evidence I am content to treat them as separate species.

Thomsonaria kohimensis (Watt ex Brandis) Rushforth. **Comb. nov.** —*Pyrus kohimensis* Watt ex Brandis. Indian trees 292 [1906]. This is named from Kohima in Nagaland, India. Aldosoro *et al* extend the range further to the East, to Cambodia, and to the West, to the Siang valley in Arunachal Pradesh and Medog district in SE Tibet, citing the following four species as synonyms. The type, Watt 7341, has 15 stamens and a fruit suggesting an affinity more with the *T. granulosa* cluster of taxa. Accordingly I do not find treating these as synonyms convincing, and, noting Stephen Spongberg' comment (Flora of China Rosaceae 9: 123 (Lu & Spongberg, 2003)) that *T. paniculata* is better associated with *T. globosa* and the treatment of *T. ochracea* and *T. subochracea* as species in the Flora of China account, I propose the following new combinations:

Thomsonaria paniculata (T. T. Yu & Tsai) Rushforth. **Comb. nov.** —*Sorbus paniculata* T. T. Yu & Tsai. Bull. Fan Mem. Inst. Biol. Bot. 7: 122 [1936]. This species is recorded from North West Yunnan.

Thomsonaria candidissima (A. Chevalier) Rushforth. **Comb. nov.** —*Pyrus candidissima* A. Chevalier. Rev. Bot. Appl. Agric. Trop. 23: 374 [1942]. This tree is described from Cambodia.

Thomsonaria ochracea (Hand.-Mazz.) Rushforth. **Comb. nov.** —*Eriobotrya ochracea* Hand.-Mazz. Symb. Sin. 7: 476 [1933]. This was named from the Yunnan-Burma border, from relatively low altitude. The iso-type of Forrest 21076 at the British Museum Natural History (BM) is in new foliage and flower and shows a relationship with *T. astaterea* rather than with *T. kohimensis.*

Thomsonaria subochracea (T. T. Yu & L. T. Lu) Rushforth. **Comb. nov.** —*Sorbus subochracea* T. T. Yu & L. T. Lu. Acta Phytotax. Sin. 18(4): 494 [1980]. This was named from specimens from Medog, South East Tibet.

In the cladogram in Guo *et al* the five samples here listed in *Thomsonaria* come out in one larger clade, albeit along with the two samples listed as "*rhamnoides*" – which I suspect have nothing to do with the Himalayan species of that name but probably also belong in *Thomsonaria.*

Dunnaria Rushforth. **Genus nov.** Type species *Dunnaria dunnii* (Rehder) Rushforth
This genus is characterised by the paired superposed ovules set in two carpels, the styles joined at the base, the oblate or sub-globose fruit which is generally wider than long, rarely globose, and small at 5—8mm in diameter. The fruit has few lenticels, or sometimes none, and ripens to red. The calyx is deciduous, leaving a nearly closed pit at the top of the fruit. The leaves are tomentose beneath, sometimes rufous hairy on the main veins below, with craspedodromus venation. The deciduous calyx, paired carpels and fruit ripening to red suggests an affinity with *Alniaria.* However, the flowers have smaller rather incurved petals and the oblate fruit, the superposed ovules which developed into a seed c. 4mm x 2.5mm x 1mm with an angled margin (and thus not the effectively nearly rounded seed in trans-section) and the craspedodromus venation show it does not belong in *Alniaria.* I note, however, that in the cladogram in Guo *et al* the sample listed as "*dunii*" came in the same clade with some *Alniaria* taxa included in their study. From *Griffitharia* the

small fruits ripening to red and the deciduous calyx, showing a greater degree of enclosure of the ovary, differentiate it.
I conclude, therefore, that it is better placed in its own genus.

Dunniaria dunnii (Rehder) Rushforth. **Comb. nov.**—*Sorbus dunnii* Rehder. Plantae Wilsonianae 2: 273 [1915]. Syn. *Pyrus koehnei* Leveille [1912], non C. K. Schneider [1906]. This tree is recorded from South East Anhui, West Zhejiang, North West Fujian, North East Guangxi, South East Guizhou and North East Yunnan.

Wilsonaria Rushforth **Genus nov.** Type species *Wilsonaria megalocarpa* (Rehder) Rushforth.
As Rehder notes (1915, p 267) "*Sorbus megalocarpa* … does not seem to be closely related to any other *Sorbus*." It has rather large fruits, 20—30mm long by 15—20mm in diameter, with persistent calyx lobes, many closely set lenticels, 3 or 4 carpels each with 2 superposed ovules, flowers in terminal corymbose panicles which open before the leaves and have 20 stamens, and large, largely glabrous leaves with craspedodromus venation.

The large corymbose flowers borne before the leaves suggest an affinity with *Pleiosorbus*. However, after careful consideration I have concluded that it does not fit there for the following reasons: Although the flowers are in large corymbs, these are subtended by leaves. It is just that the leaves develop more slowly than the flowers. In *Pleiosorbus* the corymbs open in the autumn and are leafless and remain so. Rarely a bud forms at the base of the inflorescence and opens around the time the fruits are ripe—which in *Pleiosorbus* is the next autumn—but nearly always the flowering shoot dies once the fruits are shed. The fruits take only a summer to ripen which compares with the full 12 months in *Pleiosorbus*. The 20 stamens in the flowers distinguish it from *Pleiosorbus* which has many stamens, at least 25 and usually 32—50 or so. In *Pleiosorbus* the leaves are camptodromus. The calyx is persistent with spreading lobes, cf. the incurved lobes of the calyx in *P. wardii* or the deciduous calyx of *P. megacarpus*.

From *Griffitharia* and *Thomsonaria* the fruit and foliage distinguish it. Accordingly I conclude that it is better treated in its own genus.

Wilsonaria megalocarpa (Rehder) Rushforth. **Comb. nov.**—*Sorbus megalocarpa* Rehder. Plantae Wilsonianae 2: 266 [1915]. This is found in West Sichuan south towards Kunming in Yunnan.

Wilsonaria arguta (T. T. Yu) Rushforth. **Comb. nov.**—*Sorbus arguta* T. T. Yu. Acta Phytotax. Sin. 8: 223 [1963]. This is recorded as a large shrub from South Sichuan and adjacent parts of Yunnan. I have not seen adequate material of *W. arguta* and thus am accepting it as a separate species from *W. megalocarpa* on the basis of the description and treatment in the Flora of China vol 9: 117 (Lu & Spongberg, 2003).

Wilsonaria guanxianensis (T. C. Ku) Rushforth. **Comb. nov.**—*Sorbus guanxianensis* T. C. Ku. Bull. Bot. Res., Harbin 10(3): 22 [1990]. This species seems to match Rehder's *S. megalocarpa* var. *cuneata* but from observation of cultivated plants and from the description in the Flora of China vol 9: 117 (Lu & Spongberg, 2003) I think the evidence is sufficiently strong to justify treating it as additional species in *Wilsonaria*.

In Guo *et al Wilsonaria megalocarpa* comes out as a sister clade to *Thomsonaria*.

Unplaced Species

There are three species, two accepted in Flora of China vol. 9 (Lu & Spongberg, 2003), *S. rhombifolia* C. J. Qi & K. W. Liu and *S. yunnanensis* L. T. Lu and the more recently described *S. calcicola* W. B. Liao & W. Guo which I have not been able to place. They are all recorded from what I suspect is karst limestone and appear to have camptodromus venation and two carpels in the fruit which has a persistent calyx. I think it is possible that they belong to another group, but as noted earlier the above characters are also ones shown by species in the genus *Pourthiaea*.

Taxonomic changes to *Malus sensu lata.*

Regarding *Malus*, unlike the situation with *Sorbus s.l.*, the published DNA surveys do not show *Malus s.l.* occurring in several disparate clades. When you look at Li *et al* which covers the widest sampling of the whole group, this has 32 Maloid samples which together with the *Chamaemeles* sample, cluster in a single clade (Figure 1). Thus in theory keeping them all in *Malus* could be a logical option. (Qian *et al* (2006) (modified from a Chinese paper by Liang *et al*, *non vide*) includes a similar number of *Malus s.l.* (Maloids) but with no outgroup. There is no record of the origins of their samples. Li *et al* list 32 but all their samples have gene bank references rather than origins, which could mean that most of the samples are common to the two studies.)

However, the Maloid with *Chamaemeles* clade (Fig. 1) in Li *et al* is similar in shape to the adjacent clade containing the genera *Chaenomeles*, *Cotoneaster*, *Cydonia*, *Pseudocydonia*, *Cormus*, *Pyrus* and *Pourthiaea*. So, although *Malus s.l.* is presented as a single clade, this clade appears as extensive as a clade containing seven nearly universally accepted genera and which genera are generally accepted as not being close, e.g. *Chaenomeles*, *Cydonia* and *Pseudocydonia* are close, all having multiple ovules in each carpel, and *Cormus* and *Pyrus* are also usually accepted as related, *Cotoneaster* and *Pourthiaea* are not considered close to each other or these other two groups. From this I conclude that the Maloid clade in Fig 1 of Li *et al* shows evidence in support of a narrower interpretation of genera within the Maloid group.

The clade in Li *et al* Fig. 1 has a sub-clade which includes *Chamaemeles*, *Chloromeles*, *Docynia*, *Malus doumeri*, *Docyniopsis* (= *Macromeles*), *Eriolobus* and *Malus florentina*. My interpretation is that this investigation is providing strong support for separating the following genera from *Malus* as discussed below:

Eriolobus (A. P. de Candolle) M. Roemer is well supported, with *E. trilobata* (Poiret) M. Roemer. Less clear is whether *E. florentina* (Zuccagni) Stapf belongs here or in a separate genus.

Chloromeles (Decaisne) Decaisne is also well supported for the American species (apart from *M. fusca* C. K. Schneider which belongs in *Sinomalus*, see below).

Macromeles Koidzumi. Morphology and DNA supports the treatment of *tschonoskii* as being in a separate genus, with superposed ovules and only part adnation of the ovary and hypanthium. In the recent past this has been in *Docyniopsis* (C. K. Schneider) Koidzumi [1934] but earlier Koidzumi had published *Macromeles* [1930] with *tschonoskii* as the type species. It would appear that he mistakenly thought Schneider's sectional name *Docyniopsis* had priority. He also included *Pyrus prattii* Hemsley and *Pyrus yunnanensis* Franchet in his genus. However these differ significantly from *Macromeles*, and show in a separate part of the cladogram to *tschonoskii* as discussed below.

Docynia Decaisne is also supported by Li *et al*.

Li *et al* place *Malus doumeri* (Bois.) A. Chevalier as unresolved from *Docynia* and basal to *Chloromeles*. This name (as *Pyrus doumeri* Boissier) is the oldest of perhaps four or five species from South East Asia, with *Pyrus doumeri* described from Langbian, South VietNam, *Malus formosana* Kawakami & Koidzumi from Taiwan, *Malus melliana* Hand.-Mazz. and *Malus leiocalyca* S. Z. Huang both from Southern China. *Pyrus laosensis* Cardot from Laos may also belong here. The one fruit of a species in this group which I have seen suggested at least a superficial similarly to *Chloromeles* but the stamen number is higher suggesting perhaps a closer relationship to *Macromeles*. Currently I am not in a position to resolve this issue. These species are likely to key out in the above key to either *Macromeles* or *Chloromeles* but I suspect they represent a further distinct group. An affinity with *Docynia* has been suggested but the carpels have only two ovules.

The next clade to segregate in Li *et al* is one containing *M. prattii*, *M. yunnanensis*, *M. honanensis*, *M. ombrophila* and *M. kansuensis*. *M. prattii, M. ombrophila* and *M. yunnanensis* and relatives occur along the Himalayan chain as far as Bhutan as well as south along the Indo-Burman range. They clearly differ from *Macromeles* (*Docyniopsis*), where Koidzumi placed them, in the ovary being adnate to the hypanthium, the cupular cavity at the apex of the fruit and the flowers with 20 stamens. However, they differ from *Malus* in having superposed ovules (in which respect they agree with *Macromeles tschonoskii*), in the umbellate racemes and the larger number of leaf veins. My opinion is that this group is as distinct as *Eriolobus* and *Chloromeles* and best treated as a separate genus which I propose to name *Prameles* (from shortening Antwerp Pratt's name and adding the Greek name for apple - *meles*).

Prameles Rushforth. **Genus nov**. Type species *Prameles prattii* (Hemsley) Rushforth.
This genus is characterised by the fruits with five carpels, each containing two superposed ovules. The flowers and fruits are in umbellate racemes, with the pedicels (which very rarely are branched) arising along a 1—2cm length of rachis—most easily measured when in fruit from the resting bud at the base of the inflorescence. The calyx is persistent at the top of the fruit with the 5 lobes either somewhat pressed over the top of the fruit or more usually spreading as a rosette. The lobes are fleshy, white hairy on the margins (even in the otherwise glabrous *P. prattii*) and circa 2—4mm in length. The remnants of the fused styles/top of the ovary are set as a small cone in the apical pit. The seeds are somewhat flattened with at least one acute margin. The fruit has many small flat lenticels. It is globose to oval, 1.5—2cm in length and width, slightly indented at the base. The pedicels have a few lenticels. The leaves are elliptic to ovate, rounded to subcordate at the base with an acuminate to cordate apex. The veins are in 10 or so pairs which are impressed above and raised below. The venation is camptodromus with the veins anastomosing and rarely running to a tooth. The underside of the leaf varies from tomentose, with a light covering of woolly hairs over the primary, secondary and tertiary veins and lamina, to only a few hairs on the main veins. The underside of the lamina is bullulate, as in *Griffitharia wardii*.

Species of *Prameles* occur from Central China (Hubei) southwest along the Himalaya to Bhutan and SE Tibet and south along the Indo-Burman range. They occur in the warm temperate zone below species of *Griffitharia*, to which they show some similarity but never with the density of tomentum found in most *Griffitharia*.

Accordingly the following new combinations are required:
Prameles prattii (Hemsley) Rushforth. **Comb. nov**.—*Pyrus prattii* Hemsley, Bull. Misc. Inform. Kew 1895(97) 16: 1895.

Prameles yunnanensis (Franchet) Rushforth. **Comb. nov**.—*Pyrus yunnanensis* Franchet, Plantae Delavayanae 228 [1890].

Prameles ombrophila (Hand.-Mazz.) Rushforth. **Comb. nov**.—*Malus ombrophila* Handel-Mazzetti, Acad. Wiss. Wien, Math.-Naturwiss. Kl. Anz. 63: 8 [1926].

I do not consider that *M. honanensis* belongs in *Prameles*. In Li *et al M. honanensis* sits between *prattii* and *yunnanensis* on one side and *kansuensis* and *ombrophila*, whereas in Qian *et al* these are located centrally with species of section/subgenus *Sorbomalus* on both sides. My suspicion is that *M. honanensis* probably arose as a hybrid between *prattii* and *kansuensis*, as unlike all the species (including undescribed taxa) in *Prameles* it has only 3—4 carpels/styles.

Malus Miller. Typical *Malus* comprise species with a persistent calyx and paired co-lateral ovules in fruits with 5 carpels. These are listed as section *Malus* series *Malus* in Juniper & Mabberley (2006, p 184).

The remaining apples are those with a deciduous calyx.

In the main the fruits of many of these apple species with deciduous calyces, with their pairs of erect co-lateral seeds which are round in transverse section, is similar to the fruit of *Alniaria* and *Pourthiaea* (notwithstanding this having a persistent calyx). Robertson *et al* treated all the species they leave in *Malus* in one of their three subgenera of *Malus, viz* subgenera *Malus*, *Chloromeles* and *Sorbomalus*. The question, then, is whether subgenus *Sorbomalus* holds together.

Rehder (1940) divided his interpretation of *Malus* on the character of whether the leaves in bud are folded or rolled/convoluted. This results in some of the species with deciduous calyces being in section/subgenus *Malus* and others in section/subgenus *Sorbomalus*, creating some strange bedfellows. (This is effectively the treatment given in pages 184—186 in Juniper & Mabberley (2006) which has three further sections here treated as separate genera.) This includes series *Baccatae* (or section *Gymnomeles*) occurring in section *Malus* and series *Sieboldianae* and *Kansuenses* in section *Sorbomalus*. However, Qian *et al* point out that there five different forms of folding of the leaves can be detected, making the use of this feature as proposed by Rehder (1940) simplistic.

The cladograms in Qian *et al* and Li *et al* can be read to suggest treating this group as comprising more than one genus. However, I do not currently find support for treating the species with deciduous calyces in more than one group and thus my inclination at this stage is to treat them as one genus, for which the published name is *Sinomalus* Koidzumi, based on *Malus transitoria* var. *toringoides*. If the group were further divided, a new name would be needed for those keying out at couplet 48 in the above key.

Sinomalus Koidzumi. Acta Phytotaxonomica et Geobotanica 1: 11 [1932]. Type species *Sinomalus toringoides* (Rehder) Koidzumi – lectotype selected here, as Koidzumi listed this as species 1. Koidzumi established *Sinomalus* with two species, *S. toringoides* and *S. tenuifolia*. *S. tenuifolia* was based on *Crataegus tenuifolia* Komarov [1901], non Britton [1900] and was replaced by *Crataegus komarovii* Sargent, in Plantae Wilsonianae 1: 183 [1912]. In 1934 Koidzumi transferred *transitoria* and *honanensis* to *Sinomalus*. Phipps has pointed out that *toringoides* at specific level is the same as the earlier described *Pyrus bhutanica*. Koidzumi described *Sinomalus* as having 3—5 styles and carpels. Whilst 5 styles/carpels are recorded, most species appear to have 3 or 4.

The following new combinations are required:
Sinomalus bhutanica (W. W. Smith) Rushforth. **Comb. nov**.—*Pyrus bhutanica* W. W. Smith. Rec. Bot. Surv. India 4: 265 [1911]. There is a proposal to conserve *toringoides* over *bhutanica*, which if accepted will make this combination redundant. The species is recorded from Gansu and Sichuan but the type specimen of *S. bhutanica* was collected from a cultivated tree in a monastery in Lhalung, Tibet.

Sinomalus komarovii (Sargent) Rushforth. **Comb. nov**.—*Crataegus komarovii* Sargent. Plantae Wilsonianae 1: 184 [1912]. This is recorded from South Jilin and nearby North Korea.

Sinomalus baccata (L.) Rushforth. **Comb. nov**.—*Pyrus baccata* L. Systema Naturae, ed. 12 2: 344 [1767]. This species is extremely widespread according to the literature and is probably an aggregate species.

Sinomalus mandshurica (Maximowicz) Rushforth. **Comb. nov**. *Pyrus baccata* var. *mandshurica* Maximowicz, Bull. Acad. Imp. Sci. Saint-Petersbourg, ser. 3 19(2): 170 [1874]. This is recorded from Northern and North East China and adjacent Russia.

Sinomalus halliana (Koehne) Rushforth. **Comb. nov**. *Malus halliana* Koehne, Gatt. Pomac. 27 [1890]. This is recorded from Japan, Korea and Eastern China.
Sinomalus hupehensis (Pampanini) Rushforth. **Comb. nov**. *Pyrus hupehensis* Pampanini, Nuevo Giorn. Bot. Ital., n.s. 17: 291 [1910]. This species is recorded from central China. However, it occurs either as a triploid or a tetraploid so probably apomictic and likely to contain a number of microspecies.

Sinomalus pallasiana (Juzepcuk) Rushforth. **Comb. nov**. *Malus pallasiana* Juzepcuk, Fl. URSS ix 370 [1939]. This is reported from Mongolia and adjacent parts of Siberia.

Sinomalus sachalinensis (Juzepcuk) Rushforth. **Comb. nov**. *Malus sachalinensis* Juzepcuk, Fl. URSS ix 372 [1939]. This is reported from Sakhalin.

Sinomalus spontanea (Makino) Rushforth. **Comb. nov**. *Malus spontanea* Makino, Bot. Mag. (Tokyo) 28: 295 [1914]. This is recorded from Japan.

Sinomalus sieboldii (Regel) Rushforth. **Comb. nov**. *Pyrus sieboldii* Regel, Index seminum (St. Petersbourg) 51 [1858]. This is widespread across China, Korea and Japan.

Sinomalus muliensis (T. C. Ku) Rushforth. **Comb. nov**. *Malus muliensis* T. C. Ku, Acta Phytotax. Sin. 29: 83 [1991]. This is reported from around Muli in South West Sichuan.

Sinomalus daochengensis (C. L. Li) Rushforth. **Comb. nov**. *Malus daochengensis* C. L. Li, Acta Phytotax. Sin. 27: 301 [1989]. This is report from South West Sichuan and North West Yunnan.

Sinomalus jinxianensis (J. Q. Deng & J. Y. Hong) Rushforth. **Comb. nov**. *Malus jinxianensis* J. Q. Deng & J. Y. Hong, Acta Phtyotax. Sin. 25: 326 [1987]. This is only known as a cultivated tree in South Liaoning.

Sinomalus kansuensis (Batalin) Rushforth. **Comb. nov**. *Pyrus kansuensis* Batalin, Trudy Imp. S.-Peterburgsk. Bot. Sada 13: 94 [1893]. This is recorded from Central West China.

Sinomalus fusca (Raf.) Rushforth. **Comb. nov**.—*Pyrus fusca* Raf. Med Fl. 2: 254 [1830]. This is the only species in the group not from East Asia. It is found in western North America from Alaska to California.

Sinomalus sikkimensis (Wenzig) Rushforth. **Comb. nov**. *Pyrus pashia* Bucc.-Ham. ex D. Don var. *sikkimensis* Wenzig, Linnaea 38: 49 [1874]. This species from Sikkim and Bhutan is unusual in the calyx which can be fleshy and persistent or caducous on different fruits in the same cluster. The leaves are much more hairy than in any other species in the genus. Whilst clearly a wild species, at least where I have seen it in Central Bhutan, I question whether it could be the result of introgression from a species of *Prameles* into the local form of *S. baccata*.

Sinomalus rockii (Rehder) Rushforth. **Comb. nov**. *Malus rockii* Rehder, J. Arnold Arbor. 14: 206 [1933]. The type specimen was collected in flower by Joseph Rock when travelling from Xiaguan (at the southern end of the Cang shan) west towards the Mekong (Lancang) river and the fruit is unknown. A collection, CLD 1463 from the northern end of the Cang shan, shows similarity to *S. sikkimensis*. However, the synonymy given in Lu & Spongberg (2003) includes the Bhutanese form of the *S. baccata* aggregate, which differ from *S. rockii* in several characters.

Final thoughts

Any opinion has to be tentatively final - final in that it is the best scheme that can be proposed on the author's interpretation of the available evidence on which it is based but tentative in that as and when new evidence becomes available it must be reviewed. Molecular investigation has shown that the "*mega-Sorbus*" concept proposed by Wenzig and reviewed by Rehder is untenable. Fay & Christenhusz rationalise this by proposing a "*mega-Pyrus*"—ignoring that all the other genera they subsume in their concept of *Pyrus* are well supported by currently available molecular data.

The treatment of *Sorbus s.l.* proposed here, and by extension in the key to the rest of the Malinae, is based on morphological characters but is also broadly supported by the published molecular investigations. However, there are considerable gaps in our knowledge—*Thomsonaria* has scarcely featured in molecular investigations and most species remain extremely poorly known. Clearly further investigation is required here, or with the placing of the taxa associated with, or lumped with, *Pyrus doumeri*, and I hope that a consequence of this paper will be to encourage directed research.

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Evolution of dioecious/ monecious taxa in *Juniperus*, contrasted with *Cupressus*, *Hesperocyparis*, *Callitropsis* and *Xanthocyparis* (Cupressaceae)

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ABSTRACT

Dioecy and monoecy were mapped onto phylogenies of *Juniperus* and related genera. Related genera were uniformly monecious. In contrast, the direct ancestor of *Juniperus* appears to have been dioecious, because dioecy exhibits universal occurrence in sect. Caryocedrus (*J. drupacea*) and for all species of sect. Juniperus. Monoecy appears to have re-emerged in section Sabina. The re-emergence of monoecy appears to have occurred in 5 evolutionary events: in the *californica-grandis-occidentalis-osteosperma* species of the serrate leaf junipers of North America; almost universally in the smooth leaf, turbinate, 1-seeded cone, clade centered in eastern Mediterranean and central Asia; in the *excelsa* complex; in the *chinensis* complex of central Asia and China; and in the *phoenicea/ turbinata* clade of the Mediterranean region. The genus *Juniperus*, seems to run counter-current to other closely related genera (*Cupressus, Hesperocyparis, Callitropsis, Xanthocyparis*) which are uniformly monoecious. In contrast, *Juniperus*, perhaps the most recently evolved conifer, initially evolved the atypical dioecious sexual system, then later in its evolution has (re-)evolved monoecy among many phylogenetically advanced species. Published on-line www.phytologia.org *Phytologia 100(4): 248-255 (Dec 21, 2018).* ISSN 030319430.

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Recently, Walas et al. (2018) reviewed the literature on sexual systems in gymnosperms. They reported about 65% of the gymnosperm taxa were dioecious. This is in stark contrast to angiosperms where dioecy is reported at about 6% (Renner, 2014; Renner and Ricklefs, 1995; Weiblen et al., 2000).

Dioecy has, potentially, some advantages over monoecy: complete exclusion of risk of self-pollination and optimization of resources for both male and female functions (Walas, et al., 2018). However, Walas et al. (2018) note that dioecy is not so well suited as an optimal system for fixed or sedentary taxa. Monoecy can be an advantage when no other breeding partner is nearby (Charnov, 1982; Munoz-Reinoso, 2018). In addition, because only female plants produce seeds in dioecious taxa, only half of the plants in a population are producing seed compared to monecious taxa. It does seem that monecious plants may offer an advantage in colonization of new habitats, especially by long distance dispersal, as in the cases of *Juniperus* colonization on distant, isolated islands such as Bermuda, the Caribbean Islands, the Azores, Canary Islands, etc. Among gymnosperm families (Table 1), some are almost exclusively monecious (Araucariaceae, 94.6%; Pinaceae, 100%; Sciadopityaceae, 100%) or two-thirds monecious (Cupressaceae, 64.4%). However, most families (8) are almost exclusively dioecious (Table 1). The Cupressaceae has the largest number of mixed sexual systems (taxa with both monecious and dioecious plants within a species).

Two robust phylogenies of the *Callitropsis, Cupressus, Hesperocyparis, Juniperus, Xanthocyparis* complex have recently been published. One was based on 73 nuclear genes (Mao, et al. 2018) and the other utilized the complete chloroplast genome sequences (Zhu et al, 2018). This, along with the phylogeny of *Juniperus* (Adams and Schwarzbach 2013), make it now possible to place the sexual system onto phylogenetic trees to examine if the occurrences of dioecy and monoecy is correlated with phylogeny. The purpose of the present paper is to report on phylogenetic distribution of sexual systems within *Juniperus* and in a closely related group of Cupressaceae genera (*Callitropsis, Cupressus, Hesperocyparis, Juniperus* and *Xanthocyparis*).

Table 1. Distributions of monoecious, dioecious and mixed (monoecious and dioecious plants within a taxon) species in various gymnosperm families (adapted from Walas, et al. 2018).

Family	Total species reported on	Monecious	mixed (M&D)	Dioecious	% Dioecious
Araucariaceae	37	35	0	2	5.4%
Cupressaceae	135	87	8	40	29.6
Cycadaceae	107	0	0	107	100.0
Ephedraceae	54	0	0	54	100.0
Ginkgoaceae		0	0	1	100.0
Gnetaceae	39	0	0	39	100.0
Pinaceae	224	222	2	0	0.0
Podocarpaceae	178	6	3	169	94.9
Sciadopityaceae	1	1	0	0	0.0
Taxaceae	32	1	1	30	93.7
Welwitschiaceae	1	0	0	1	100.0
Zamiaceae	224	0	0	224	100.0
Total	1033	352 (34.1%)	14 (1.36%)	667 (64.56%)	

MATERIALS AND METHODS

Information on dioecy or monoecy was obtained from Adams' monograph of *Juniperus* (Adams 2014; Farjon 2005). For *Callitropsis, Cupressus, Hesperocyparis* and *Xanthocyparis,* information was from Walas et al. 2018 and Farjon 2005.

RESULTS AND DISCUSSION

Table 2 shows the classification of 119 *Juniperus* taxa as to their sexual system with 88 taxa dioecious (73.9%) and 31 taxa (26.1%) having some degree of monoecy as: 12 taxa monoecious (10.2%); 13 taxa dioecious & monoecious (10.9%); 6 taxa mostly dioecious but rarely monoecious (5.0%). Vasek (1966) made a very detailed study of thousands of specimens and natural plants to determine the frequency of dioecious vs. monecious plants within a population and taxon. So, we do have quantitative data of monoecy in *J. californica* (1.9% monecious), *J. grandis* (Vasey treated as *J. occidentalis* subsp. *australis*) (5.1%), *J. occidentalis* (47.6%) and *J. osteosperma* (89.2%).

In addition, Jordano (1991) conducted a detailed study of monoecy and sex expression in *J. phoenicea* in Spain and Morocco. He found although *J. phoenicea* is considered monoecious, in reality, most trees in a population are 'mostly with pollen cones and few seed cones' ('male plants') or 'mostly with seed cones and a few pollen cones' ('female plants') with a few plants that produced numerous pollen cones and seed cones ('true monecious' plants). This situation he called a 'functionally subdioecious breeding system'. Just in this issue of Phytologia, Munoz-Reinoso (2018) reported finding 1 monecious tree in a population of dioecious *J. oxycedrus* var. *badia* trees. For a more detailed review of sex expression in *Juniperus*, the reader is referred to Adams (2014, chapter 9). It should be noted that I have examined thousands of juniper trees in the field over the past 50 years and have encountered a few 'monecious' trees (with a few male cones, and many female cones, or many male cones and a few female seed cones) in 'dioecious' taxa of most species.

Table 2. Classification of all *Juniperus* species and varieties (119) by sexual system (dioecious, monecious or both, within a taxon). Data from Adams (2014) and Vasek (1966) for percentage monecious data for *J. californica, J. grandis* (*J. occidentalis* subsp. *australis*), *J. occidentalis* and *J. osteosperma*.

Taxon, nomenclature of Adams, 2014	Plants: dioecious, monecious or both
J. angosturana R. P. Adams	dioecious
J. arizonica (R. P. Adams) R. P. Adams	dioecious
J. ashei Buchholz	dioecious
J. barbadensis L.	dioecious
J. barbadensis var. *lucayana* (Britton) R. P. Adams	dioecious
J. bermudiana L.	dioecious
J. blancoi var. *huehuentensis* R. P. Adams, S. Gonzalez, and M. G. Elizondo	dioecious
J. blancoi var. *mucronata* (R. P. Adams) Farjon	dioecious
J. blancoi Martinez var. *blancoi*	dioecious
J. brevifolia (Seub.) Ant.	dioecious
J. californica Carriere	dioecious, rarely monecious (1.9%)
J. californica f. *lutheyana* J. T. Howell & Twisselm.	dioecious
J. carinata (Y. K. Yu & L. K. Fu) R. P. Adams	dioecious
J. cedrus Webb & Berthol.	dioecious
J. chinensis var. *procumbens* Sieb.ex Endl.	monecious
J. chinensis var. *sargentii* Henry	dioecious, rarely monecious
J. chinensis L. var. *chinensis*	dioecious
J. coahuilensis (Martinez) Gaussen ex R. P. Adams	dioecious
J. comitana Martinez	dioecious
J. communis L. var. *communis*	dioecious
J. communis var. *charlottensis* R. P. Adams	dioecious
J. communis var. *depressa* Pursh	dioecious
J. communis var. *hemisphaerica* (J. & C. Presl) Parl.	dioecious
J. communis var. *kamchatkensis* R. P. Adams	dioecious
J. communis var. *kelleyi* R. P. Adams	dioecious
J. communis var. *megistocarpa* Fernald & H. St. John	dioecious
J. communis var. *nipponica* (Maxim.) E. H. Wilson	dioecious
J. communis var. *saxatilis* Pall. (only in eastern hemisphere)	dioecious
J. convallium f. *pendula* (Cheng & L. K. Fu) R. P. Adams	dioecious & monecious
J. convallium Rehder & Wilson	dioecious & monecious
J. coxii A.B. Jacks	dioecious
J. davurica var. *arenaria* (E. H. Wilson) R. P. Adams	dioecious
J. davurica var. *mongolensis* (R. P. Adams) R. P. Adams	dioecious
J. davurica Pall.	dioecious
J. deltoides R. P. Adams	dioecious
J. deltoides var. *spilinanus* (Yalt., Elicin & Terzioglu) Terzioglu	dioecious
J. deppeana var. *patoniana* (Martinez) Zanoni	dioecious
J. deppeana var. *robusta* Martinez	dioecious
J. deppeana forma *elongata* R. P. Adams	dioecious
J. deppeana forma *sperryi* (Correll) R. P. Adams	dioecious
J. deppeana forma *zacatacensis* (Mart.) R. P. Adams	dioecious
J. deppeana Steudel var. *deppeana*	dioecious
J. deppeana var. *gamboana* (Mart.) R. P. Adams	dioecious, rarely monecious
J. drupacea Labill.	dioecious
J. durangensis var. *topiensis* R. P. Adams & S. Gonzalez	dioecious

J. durangensis Martinez	dioecious
J. erectopatens (Cheng & L. K. Fu) R. P. Adams	dioecious
J. excelsa M.-Bieb. var. excelsa	dioecious & monecious
J. fargesii (Rehder & Wils.) Kom.	dioecious
J. flaccida Schlecht.	dioecious
J. foetidissima Willd.	dioecious & monecious
J. formosana Hayata	dioecious
J. gracilior Pilger	dioecious
J. gracilior var. *ekmanii* (Florin) R. P. Adams	dioecious
J. gracilior var. *saxicola* (Britton & P. Wilson) R. P. Adams	dioecious
J. gracilior var. *urbaniana* (Pilger & Ekman) R .P. Adams	dioecious
J. grandis R. P. Adams	dioecious, 5.1% monecious
J. horizontalis Moench	dioecious
J. indica var. *caespitosa* Farjon	dioecious & monecious
J. indica Bertol.	dioecious & monecious
J. jackii (Rehder) R. P. Adams	dioecious
J. jaliscana Martinez	dioecious
J. komarovii Florin	dioecious & monecious
J. macrocarpa Sibth. & Sm.	dioecious
J. maderensis (Menezes) R. P. Adams	dioecious
J. mairei Lemee & Lev.	dioecious
J. maritima R. P. Adams	dioecious
J. martinezii Perez de la Rosa	dioecious
J. microsperma (Cheng & L. K. Fu) R. P. Adams	dioecious & monecious
J. monosperma (Engelm.) Sarg.	dioecious
J. monticola forma *compacta* Martinez	dioecious
J. monticola forma *orizabensis* Martinez	dioecious
J. monticola Martinez forma *monticola*	dioecious
J. morrisonicola Hayata	dioecious & monecious
J. navicularis Gand.	dioecious
J. occidentalis f. *corbetii* R. P. Adams	dioecious & monecious
J. occidentalis Hook.	dioecious, 52.4% & monecious, 47.6%
J. osteosperma (Torr.) Little	monecious, 89.2%, seldom dioecious, 10.8%
J. ovata (R. P. Adams) R. P. Adams	dioecious
J. oxycedrus L.	dioecious
J. phoenicea L.	monecious, rarely dioecious
J. pinchotii Sudworth	dioecious
J. pingii Cheng & Ferre.	monecious
J. pingii var. *miehei* Farjon	monecious
J. poblana (Martinez) R. P. Adams	dioecious
J. poblana var. *decurrens* R. P. Adams	dioecious
J. polycarpos K. Koch	dioecious
J. polycarpos var. *turcomanica* (B. Fedtsch.) R. P. Adams	dioecious
J. procera Hochst. ex. Endl.	dioecious & monecious
J. przewalskii Kom.	monecious
J. pseudosabina Fisch., Mey. & Ave-Lall.	dioecious, rarely monecious
J. recurva Buch.-Ham. ex D. Don.	monecious, rarely dioecious
J. rigida var. *conferta* (Parl.) Patschka	dioecious
J. rigida Mig. in Sieb. var. *rigida*	dioecious
J. rushforthiana (R. P. Adams) R. P. Adams	monecious
J. sabina L.	dioecious, rarely monecious
J. sabina var. *balkanensis* R. P. Adams & A. N. Tashev	dioecious, rarely monecious

J. saltillensis M. T. Hall	dioecious
J. saltuaria Rehder & Wils.	monecious
J. scopulorum Sarg.	dioecious
J. semiglobosa Regel var. *semiglobosa*	dioecious
J. semiglobosa var. *jarkendensis* (Kom.) R. P. Adams	dioecious
J. semiglobosa var. *talassica* (Lipsky) Silba	dioecious
J. seravschanica Kom.	dioecious
J. squamata var. *wilsonii* (Rehder) R. P. Adams	dioecious
J. squamata Buch.-Ham. ex D. Don in Lambert	monecious, rarely dioecious
J. standleyi Steyermark	dioecious
J. taxifolia Hook. & Arn.	dioecious
J. taxifolia var. *lutchuensis* (Koidz.) Satake	dioecious
J. thurifera L. var. *thurifera*	dioecious
J. thurifera var. *africana* Maire	dioecious
J. tibetica Kom.	monecious
J. tsukusiensis var. *taiwanensis* (R. P. Adams & C-F. Hsieh) R. P. Adams	dioecious
J. tsukusiensis var. *tsukusiensis* Masam.	dioecious
J. turbinata Guss.	dioecious & monecious
J. uncinata (R. P. Adams) R. P. Adams	monecious, rarely dioecious
J. virginiana L. var. *virginiana*	dioecious
J. virginiana var. *silicicola* (Small) E. Murray	dioecious
J. zanonii R. P. Adams	dioecious
Summary: completely 88 dioecious (74.8%); Not completely dioecious 31 (25.2%): 12 monoecious (10.2%); 13 dioecious & monoecious (10.9%); 6 mostly dioecious, rarely monoecious (5.0%)	

To examine the phylogenetic distribution of dioecious and monoecious taxa, these traits were mapped onto a phylogenetic tree (Adams, 2014; Adams and Schwarzbach, 2013) shown in figure 1. The incidence of dioecy groups by clades (Fig. 1): all of sect. Caryocedrus and sect. *Juniperus* are dioecious; monoecy is only found in sect. Sabina, and even there, monoecy is grouped by clades. The serrate leaf margined junipers of the western hemisphere (percent data from Vasek, 1966) have 4 taxa with monoecy: *J. californica* (rarely monecious, 1.9%), *J. grandis* (5.1% monecious), *J. occidentalis*, 47.6% monecious) and *J. osteosperma* (89.2% monecious). This appears to be an isolated case of monoecy among the dioecious taxa of the serrate *Juniperus* of the western hemisphere (Fig. 1). The clade of smooth leaf, turbinate, 1-seeded cones, junipers of the eastern hemisphere (India to western China to Taiwan), contain only 3 taxa that appear to be dioecious: *J. fargesii*, *J. carinata*, and *J. coxii* and these are uncertain, because of limited observations of plants in the field by the author. They may be partially monecious. Fifteen (15) of the 18 taxa in this clade are monecious or partially monecious (Fig. 1). The third case of monoecy is in the excelsa group, smooth leaves, ovoid, multi-seeded cones, eastern hemisphere (eastern Mediterranean to western China, and Japan). This clade is composed a sub-clade of the *virginiana* group in the western hemisphere, that are all dioecious and a loose assemblage of junipers, of which approximately half are monoecious taxa (Fig. 1).

Finally, the fourth clade with monoecy is the *phoenicea*/ *turbinata* clade in the Mediterranean (Fig. 1). Both taxa in this clade have considerable monoecy. It might be noted *Hesperocyparis arizonica* and *H. bakeri* (outgroup) are both monecious.

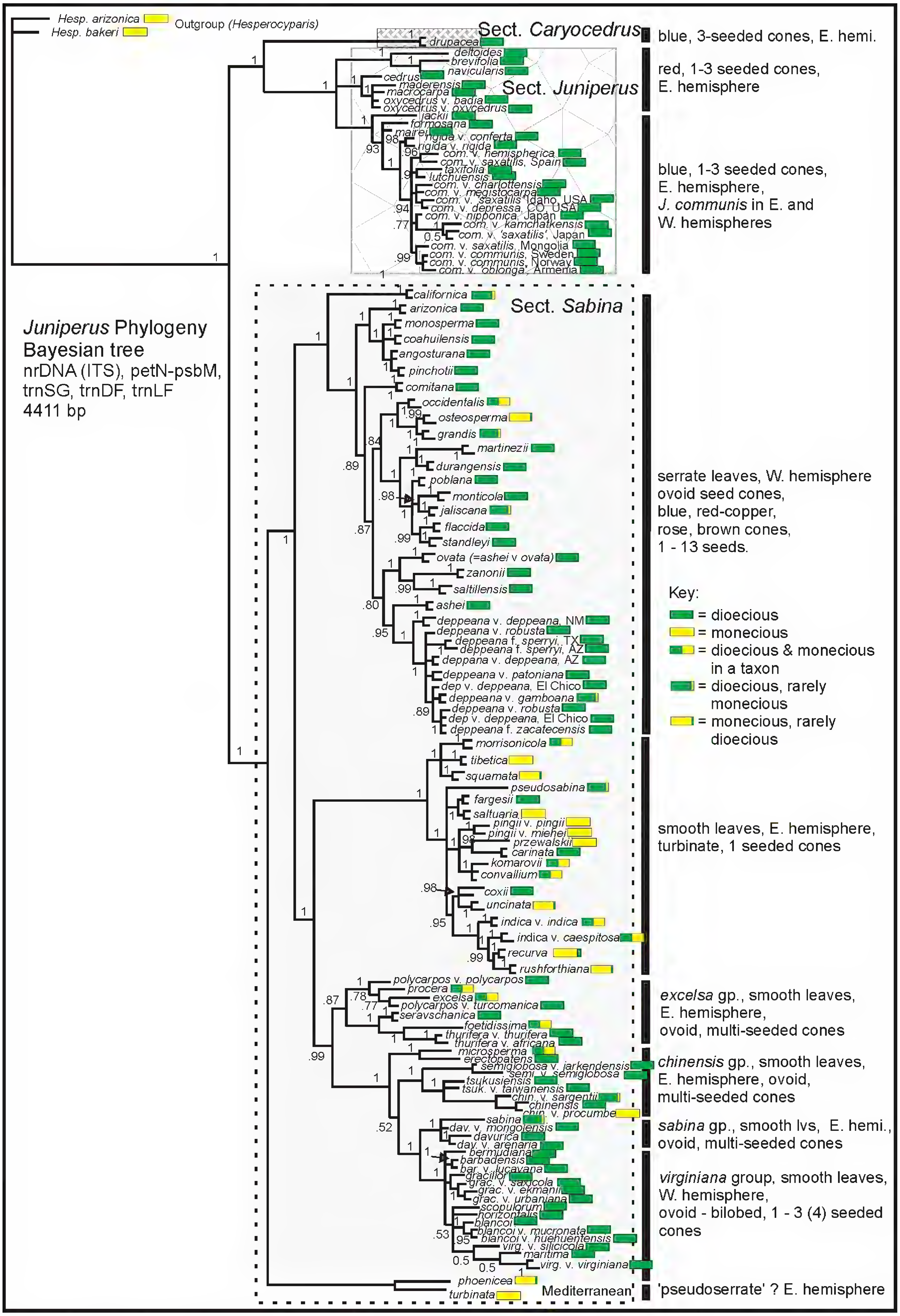

Figure 1. Bayesian phylogenetic tree of *Juniperus* with sexual system imposed.

All of the closely related relatives of *Juniperus* (Mao, et al. 2018; Zhu et al, 2018) are monecious (Table 2). This is also true (Fig. 2) for distant relatives (*Calocedrus, Microbiota, Platycladus, Thuja*).

Table 2. Sexual systems in cypresses (data ex Farjon, 2005; Rushforth, pers. comm.; Walas et al. 2018).

Taxon	Plants: dioecious, monecious or both; ex Farjon, 2005; Walas et al. 2018.
Callitropsis nootkatensis	monecious
Cupressus cashmeriana	monecious
Cupressus chengiana	monecious
Cupressus duclouxiana	monecious
Cupressus dupreziana	monecious
Cupressus funebris	monecious
Cupressus gigantea	monecious
Cupressus sempervirens	monecious
Cupressus tonkinensis	monecious
Cupressus torulosa	monecious
Hesperocyparis arizonica	monecious
Hesperocyparis bakeri	monecious
Hesperocyparis benthamii	monecious
Hesperocyparis glabra	monecious
Hesperocyparis goveniana	monecious
Hesperocyparis guadalupensis	monecious
Hesperocyparis lusitanica	monecious
Hesperocyparis macnabiana	monecious
Hesperocyparis macrocarpa	monecious
Hesperocyparis sargentii	monecious
Xanthocyparis vietnamensis	monecious

Plotting the sexual systems onto phylogenies of Mao, et al. 2018 and Zhu et al, 2018, clearly suggests that ancestors of *Juniperus* were monecious (yellow boxes, Figs.1,2). However, the direct ancestor of *Juniperus* appears to have been dioecious (green boxes, Figs. 1,2), because dioecy exhibits

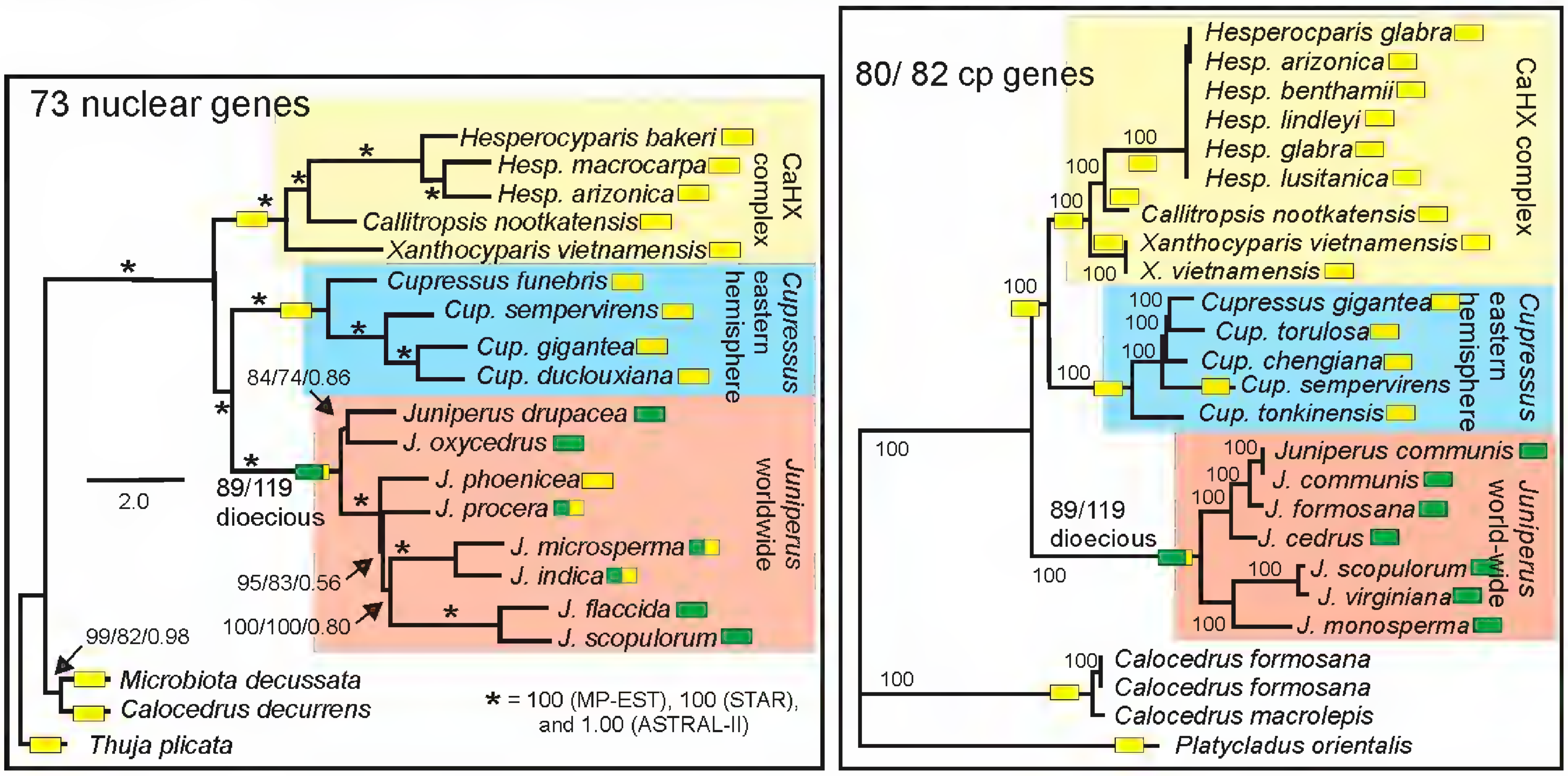

Figure 2. (left) Phylogeny of cypress-juniper clade based on 73 nuclear genes (Mao et al. 2018) with dioecy (green) and monoecy (yellow) included. (right) Phylogeny based on complete chloroplast genome sequencing (Zhu, et al. 2018) with dioecy and monoecy included. See text for discussion.

universal occurrence in sect. Caryocedrus (*J. drupacea*) and for all species of sect. Juniperus (*J. communis* and related species, Figs. 1, 2). Section Sabina is considered the most advanced section of *Juniperus*, and it is there, that one finds the re-emergence of monoecy.

The re-emergence of monoecy appears to have occurred in 5 events: in the *californica-grandis-occidentalis-osteosperma* species of the serrate leaf junipers of North America; almost universally in the smooth leaf, turbinate, 1-seeded cone, clade centered in eastern Mediterranean and central Asia; in the *excelsa* and *chinensis* complexes of central Asia and China; and in the *phoenicea/ turbinata* clade of the Mediterranean region.

Walas et al. (Table 3, 2018) reported that dioecy was more common in tropical gymnosperms whereas monoecy was more common colder climate gymnosperms. This does not appear to be the case in *Juniperus* in which cold-tolerant species are mostly dioecious.

In summary, the genus *Juniperus*, seems to run counter-current to other closely related genera (*Cupressus, Hesperocyparis, Callitropsis, Xanthocyparis*) which are uniformly monoecious. In contrast, *Juniperus*, perhaps the most recently evolved conifer, with the evolution of nutritious, small cones that are easily dispersed by birds over long distances, initially evolved the atypical dioecious sexual system, then later in its evolution has (re-) evolved monoecy among many phylogenetically advanced species.

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New Combinations in Parasenegallia and Mariosousa (Fabaceae: Mimosoideae)

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ABSTRACT

Morphological and genetic differences separating the subgenera of *Acacia* s. l., and molecular evidence that the genus *Acacia* s.l. is polyphyletic, necessitate transfer of species from *Senegalia* to *Parasenegalia*, resulting in four new combinations: ***Parasenegalia amorimii*** (M. J. F. Barros & M. P. Morim) Seigler & Ebinger, **comb. nov.**, ***P. grazielae*** (M. J. F. Barros & M. P. Morim) Seigler & Ebinger,, **comb. nov.**, ***P. incerta*** (Hoehne) Seigler & Ebinger, **comb. nov.**, and ***P. miersii*** (Benth.) Seigler & Ebinger, **comb. nov.** The names *Acacia incerta* and *A. miersii* are lectotypified. **Mariosousa heterophylla** (Benth.) Seigler & Ebinger, **comb. nov.** is necessary for the transfer of *Prosopis heterophylla* Benth. to the genus *Mariosousa*. Published on-line www.phytologia.org *Phytologia 100(4): 256-259 (Dec 21, 2018).* ISSN 030319430.

KEY WORDS: *Acacia* sensu lato, Fabaceae, lectotype, *Mariosousa*, Mimosoideae, new combination; *Parasenegalia*, *Senegalia*.

Morphological differences separating species of *Senegalia* Rafinesque (1838) and molecular evidence that the genus *Senegalia* is polyphyletic (cf. Miller & Seigler, 2012) necessitate transfer of certain species to the genus *Parasenegalia* Seigler & Ebinger in Seigler et al. (2017). For the following four taxa, this results in their transfer to the segegrate genus *Parasenegalia* (Seigler et al., 2017):

1. **PARASENEGALIA AMORIMII** (M. J. F. Barros & M. P. Morim) Seigler & Ebinger, *comb. nov.* Basionym: *Senegalia amorimii* M. J. F. Barros & M. P. Morim, Syst. Bot. 39(2): 454, 456, Fig. 1. 2014. – TYPE: BRAZIL. BAHIA: "Porto Seguro. Reserva da Brasil Holanda de Ind. S/A. Entrada no Km. 22 da Rod. Eunápolis/P. Seguro. Ca. 9.5 km na entrada." 16° 27' 45" S, 39° 19' 31" W, s. d., fl., *A. M. de Carvalho n°. 4499, with A. M. Amorim, S. C. Sant'Ana*, & *J. G. Jardim* (holotype: RB, RB digital image at CEPEC).

Although described originally in *Senegalia* (Barros, 2011; Barros & Morim, 2014), the complete absence of prickles indicates that this species should be transferred to *Parasenegalia*).

2. **PARASENEGALIA GRAZIELAE** (M. J. F. Barros & M. P. Morim) Seigler & Ebinger, *comb. nov.* Basionym: *Senegalia grazielae* M. J. F. Barros & M. P. Morim, Syst. Bot. 39(2): 461-462, Fig. 5. 2014. – TYPE: BRAZIL. ESPÍRITO SANTO: Águia Branca, Rochedo," 18° 56' 40.4" S, 40° 48' 7" W, 19 Dec. 2007, fl. *V. Demuner 4783*, *T. A. Cruz* & *M. Belisário* (holotype: RB; isotypes: HUEFS, MBML).

Although described originally in *Senegalia* (Barros, 2011; Barros & Morim, 2014), the complete absence of prickles indicates that this species should be transferred to *Parasenegalia*).

3. **PARASENEGALIA INCERTA** (Hoehne) Seigler & Ebinger, *comb. nov*. Basionym: *Acacia incerta* Hoehne, Com. Lin. Telegr., Bot., 45(8): 22-23, pl. 135. 1919. *Senegalia incerta* (Hoehne) Seigler & Ebinger, Phytologia 92(1): 93. 2010. – TYPE: BRAZIL. MATO GROSSO: Cuiabá, Coxipó da Ponte, 1911, *F. C. Hoehne* [CLTE] *2535* (lectotype, here designated, R-19882 [barcode] R000019882).

A syntype 2534 was also found at the Museo National in Rio de Janeiro. Both numbers 2534 and 2535 represented collections made for the Commissão de Linhas Telegraphicas Estratégics de Mato Grosso ao Amazonas (CLTE) and were not personal collection numbers (M. C. Mamede, personal communication).

4. **PARASENEGALIA MIERSII** (Benth.) Seigler & Ebinger, *comb. nov.* Basionym: *Acacia miersii* Benth., London J. Bot. 1: 522. 1842. *Senegalia miersii* (Benth.) Seigler & Ebinger in Seigler et al. Phytologia 88(1): 59. 2006. – TYPE: BRAZIL. RIO DE JANEIRO: "Corcovado," *J. Miers 3864* (lectotype, here designated, K [barcode] K000530839).

The taxon originally described by Bentham (1842) as *Acacia miersii* was stated to be from "Aqueduct of Rio Janeiro" in the protologue. Material of this collection has not been relocated. Another collection (*J. Miers 3864*) was considered to be type material (L. Rico annotation). However, the specimen has "hort. Petrop." and "Luschnat" written on the sheet and this may represent a duplicate that was transferred to K at some time in the past. Bentham (1876) cited "habitat in monte Corcovado prope Rio de Janeiro: Miers, Luschnath" and a Schott collection from Serra Tingua. There is nothing on the specimen itself that indicates that it was seen by Bentham. For that reason (Art. 40, especially Note 1), we choose to lectotypify *J. Miers 3864*.

A taxon originally described by Bentham (1846) in *Prosopis* is transferred here to *Mariosousa* Seigler & Ebinger (Seigler et al., 2006). The two genera are contrasted by the absence of spines in *Mariosousa*, by the stamen number (multiple, approximately 100 in *M. heterophylla* vs. ten stamens in *Prosopis*, and by the fruits in cross-section (flattened in *Mariosousa* vs. oval to flattened in *Prosopis*). A proposal to conserve the name *Acacia willardiana* Rose against *Prosopis heterophylla* Benth. (Seigler & Ebinger, 2008) was declined (Brummitt, 2011; Barrie, 2011).

5. **MARIOSOUSA HETEROPHYLLA** (Benth.) Seigler & Ebinger, *comb. nov*. Basionym: *Prosopis heterophylla* Benth., London J. Bot. 5: 82. 1846, as "*P. ? heterophylla.*". *Senegalia heterophylla* (Benth.) Britton & Rose, N. Amer. Fl. 23(2): 114. 1928. -- TYPE: MEXICO. SONORA. "Sonora alta," 1830, *T. Coulter s.n.*(holotype, TCD). [= *Acacia willardiana* Rose in Vasey & Rose, 1890; = *Mariosousa willardiana* (Rose) Seigler & Ebinger in Seigler et al., 2006.]

Key to taxa affined to *Senegalia* s.l.:

1. Prickles common on stem and usually the leaf petiole and rachis* . . . . *Senegalia*
1. Prickles absent on stem, leaf petiole and rachis.
   2. Inflorescence a globose to subglobose head (as broad as long or nearly so).
      3. Flowers pedicellate; stamens consistently 200+; petiolar glands absent . . . . *Acaciella*
      3. Flowers sessile to rarely subsessile; stamens less than 200; petiolar glands present . . . . *Parasenegalia*, pro parte
   2. Inflorescence a cylindrical spike (commonly twice or more longer than wide)
      4. Leaflets small, 1.2-2.9 mm long and 0.4-0.9 mm wide; petioles 0.5-13 mm long; short shoots present.

5. Perianth scarious; flowers 7.5-10.0 mm long, the corolla 3.3-6.0 mm
Long (Bolivia) . . . . *Pseudosenegalia*
5. Perianth not scarious; flowers 5.5-7.5 mm long, the corolla 2-3 mm
long (Oaxaca & Puebla, Mexico) . . *Mariosousa compacta* (Rose) Seigler & Ebinger
4. Leaflets mostly longer and wider; petioles mostly more than 15 mm long;
short shoots absent.
6. Fruit valves coriaceous; lenticels on twigs orange, orbicular to slightly
elongated vertically, some more than 0.6 mm across . . . . *Parasenegalia*, pro parte
6. Fruit valves thinner, chartaceous; lenticels not orange, orbicular to
slightly elongated horizontally, very rarely to 0.6 mm across . . . . *Mariosousa*

*Although the prickles that are characteristic for *Senegalia* may appear to be absent on rare individual specimens, careful examination usually reveals poorly developed prickles or small enations on the stems, petiole or rachis of the specimens.

Key to the species of *Pseudosenegalia* and *Parasenegalia*:
1. Inflorescence a cylindrical spike.
2. Leaflets 1.2-2.9 mm long; perianth scarious; short shoots present below many
of the nodes (Bolivia).
3. Leaves with a single pair of pinna; distance between leaflet pairs 0.3-0.6 mm . . . . . .
. . . . *Pseudosenegalia feddeana* (Harms) Seigler & Ebinger
3. Leaves with two or more pinna pairs; distance between leaflet pairs
0.8-1.7 mm . . Pseudo*senegalia riograndensis* (Atahuachi & L. Rico) Seigler & Ebinger
2. Leaflets more than 4.0 mm long; perianth not scarious; short shoots absent.
4. Leaflets 30 to 65 pairs/pinna; 0.7-1.6 mm between leaflet pairs (Caribbean) . . . .
. . . . *Parasenegalia skleroxyla* (Tussac) Seigler & Ebinger
4. Leaflets less than 30 pairs/pinna; 1.4 mm or more between leaflet pairs.
5. Leaflets 1.8-3.2 mm wide; 1.4-4.0 mm between leaflet pairs (Belize,
Guatemala) . . . . *Parasenegalia lundellii* Seigler & Ebinger
5. Leaflets 4.0-11.3 mm wide; 3.8-9.5 mm between leaflet pairs (Caribbean) . . . . . .
. . . . *Parasenegalia muricata* (L.) Seigler & Ebinger
1. Inflorescence a globose to subglobose head.
6. Leaflets 25-100 mm long; 1 to 2 pinna pairs/leaf (Brazil) . . . .
. . . . *Parasenegalia miersii* (Benth.) Seigler & Ebinger
6. Leaflets less than 24 mm long; 3 or more pinna pairs/leaf
7. Petioles 2-17 mm long.
8. Petioles 3-7 mm long; inflorescences 10-14 mm across (Brazil) . . . .
. . . . *Parasenegalia santosii* (G. P. Lewis) Seigler & Ebinger
8. Petioles 13-16 mm long; inflorescences 6-8 mm across (Brazil) . . . .
. . . . . *Parasenegalia grazielae* (M. J. F. Barros & M. P. Morim) Seigler & Ebinger
7. Petioles more than 18 mm long.
9. Pinna pairs/leaf 15 to 20 (Brazil) *Parasenegalia incerta* (Hoehne) Seigler & Ebinger
9. Pinna pairs/leaf 3 to 14.
10. Inflorescence 16-23 mm across; leaflets 0.8-2.1 mm wide, the midvein
bluish purple beneath (Argentina, Bolivia, Chile, Peru, Venezuela) . . . .
. . . . *Parasenegalia visco* (Lorentz ex Griseb.) Seigler & Ebinger
10. Inflorescences 4-10 mm across; leaflets 2-9 mm wide, the midvein
not bluish-purple beneath.
11. Leaflets 5-9 mm long and 2-3 mm wide; inflorescence 4-7 mm
across; petiolar gland present on the lower half of the petiole (Brazil) . . . . .
. ..*Parasenegalia amorimii* (M. J. F. Barros & M. P. Morim) Seigler & Ebinger
11. Leaflets 7-18 mm long and 2.3-8.1 mm wide; inflorescence 6.5-14 mm

across; petiolar gland usually just below the lowermost pinna pair.

12. Inflorescences 6-10 mm across; 6 to 9 pinna pairs/leaf (Bolivia, Peru) . . . . *Parasenegalia rurrenabaqueana* (Rusby) Seigler & Ebinger
12. Inflorescences 9-14 mm across; 3 to 6(7) pinna pairs/leaf (Caribbean) . . . . . *Parasenegalia vogeliana* (Steud.) Seigler & Ebinger

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